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(71) Applicant: SCHERING CORPORATION [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033 (US).			
(72) Inventors: GISH, Kurt, C.; 974 Hayes Street, San Francisco, CA 94117 (US). SCHALL, Thomas, J.; 2050 Mills Avenue, Menlo Park, CA 94025 (US). VICARI, Alain; 2250 Latham Street #54, Mountain View, CA 94087 (US). ZLOTNIK, Albert; 507 Alger Drive, Palo Alto, CA 94306 (US). WANG, Wei; 3090 Middlefield Road, Palo Alto, CA 94306 (US).			
(74) Agents: DULAK, Norman, C. et al.; Schering-Plough Corporation, Patent Dept. K-6-1 1990, 2000 Galloping Hill Road, Kenilworth, NJ 07033-0530 (US).			

(54) Title: MAMMALIAN CHEMOKINE REAGENTS

(57) Abstract

Novel chemokines from mammals, reagents related thereto including purified proteins, specific antibodies, and nucleic acids encoding said chemokines. Chemokine receptors are also provided. Methods of using said reagents and diagnostic kits are also provided.

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MAMMALIAN CHEMOKINE REAGENTS

FIELD OF THE INVENTION

The present invention relates to compositions related to proteins which function in controlling physiology, development, and/or differentiation of mammalian cells, e.g., cells of a mammalian immune system. In particular, it provides proteins and mimetics that regulate physiology, development, differentiation, and function of various cell types, including hematopoietic cells. It also provides receptor reagents for chemokine-like proteins.

BACKGROUND OF THE INVENTION

The circulating component of the mammalian circulatory system comprises various cell types, including red and white blood cells of the erythroid or the myeloid cell lineages. See, e.g., Rapaport (1987) Introduction to Hematology (2d ed.) Lippincott, Philadelphia, PA; Jandl (1987) Blood: Textbook of Hematology, Little, Brown and Co., Boston, MA.; and Paul (ed.) (1993) Fundamental Immunology 3d ed., Raven Press, N.Y. Progression through various stages of differentiation is regulated by various signals provided to the cells, often mediated through a class of proteins known as the cytokines. Within this group of molecules as a further group known as the chemoattractant cytokines, or chemokines. See, e.g., Schall (1994) "The Chemokines" in Thomson (ed.) The Cytokine Handbook (2d ed.) Academic Press; and Schall and Bacon (1994) Current Opinion in Immunology 6:865-873.

Although the full spectrum of biological activities of the chemokines has not been extensively investigated, chemoattractant effects are recognized. The best known biological functions of these molecules relate to

chemoattraction of leukocytes. However, new chemokines are being discovered, and their biological effects on the various cells responsible for immunological responses are topics of continued study.

5 Certain chemokine receptors have also been characterized. See, e.g., Samson, et al. (1996) Biochemistry 35:3362-3367; and Rapport, et al. (1996) J. Leukocyte Biology 59:18-23.

10 These observations indicate that other factors exist whose functions in hematopoiesis, immune development, and leukocyte trafficking were heretofore unrecognized. These factors provide for biological activities whose spectra of effects are distinct from known differentiation, activation, or other signaling factors.

15 The absence of knowledge about the structural, biological, and physiological properties of the regulatory factors which regulate hematopoietic cell physiology in vivo prevents the modification of the effects of such factors. Thus, medical conditions where

20 regulation of the development or physiology of relevant cells is required remains unmanageable.

SUMMARY OF THE INVENTION

The present invention is based, in part, upon the discovery of new genes encoding chemokines, and new genes encoding various receptors for chemokines. It embraces agonists and antagonists of the chemokines. In particular, sequences of various chemokines, e.g., designated Thymus Expressed ChemoKine (TECK); MIP-3 α ; MIP-3 β ; and chemokine receptors designated "dendritic cell receptor for chemokine" (DC CR) and "monocyte/dendritic cell receptor for chemokine" (M/DC CR); and mutations (muteins) of the respective natural sequences, fusion proteins, chemical mimetics, antibodies, and other structural or functional analogs are provided. It is also directed to isolated genes encoding respective proteins of the invention. Various uses of these different protein or nucleic acid compositions are also provided.

The present invention provides a substantially pure or isolated polypeptide comprising a segment exhibiting sequence homology to a corresponding portion of a mature TECK, MIP-3 α , MIP-3 β , DC CR, or M/DC CR, wherein the

5 homology is at least about 70% identity and the portion is at least about 25 amino acids. Preferably, the protein further comprises a second segment exhibiting at least about 90% identity over at least 9 amino acids; or at least about 80% identity over at least 17 amino acids.

10 In other preferred embodiments, the polypeptide: is from a warm blooded animal selected from the group of birds and mammals, including a mouse or human; comprises a natural sequence set forth in SEQ ID NO: 2, 4, 6, 8, 10 or 12; exhibits a post-translational modification pattern

15 distinct from a natural form of the polypeptide; is made by expression of a recombinant nucleic acid; comprises synthetic sequence; is detectably labeled; is conjugated to a solid substrate; is conjugated to another chemical moiety; is a fusion protein; is in a denatured

20 conformation, including detergent denaturation; further comprises an epitope tag; is an immunogenic polypeptide; has a defined homogeneous molecular weight; is useful as a carbon source; is an allelic variant of SEQ ID NO: 2, 4, 6, 8, 10, or 12; is a 3-fold or less substituted form

25 of a natural sequence; is in a sterile composition; is in a buffered solution or suspension; is in a regulated release device; comprises a post-translational modification; is in a cell; or is in a kit which further comprises instructions for use or disposal of reagents

30 therein.

In other aspects, the invention provides an isolated or recombinant nucleic acid encoding such protein, where the portion consists of sequence from the coding region of SEQ ID NO: 1, 3, 5, 7, 9, or 11. Other aspects

35 include such nucleic acids which: exhibit at least about 80% identity to a natural cDNA encoding said segment; is in an expression vector; further comprises a promoter; further comprises an origin of replication; is from a natural source; is detectably labeled; comprises

synthetic nucleotide sequence; is less than 6 kb; is from a mammal; comprises a natural full length mature coding sequence; is in a kit, which also comprises instructions for use or disposal of reagents therein; is a specific hybridization probe for a gene encoding the protein; is a PCR product; or is in a cell. The invention also provides a method of using a purified nucleic acid by expressing the nucleic acid to produce a protein.

Alternatively, the invention provides an isolated or recombinant nucleic acid which encodes at least eight consecutive residues of SEQ ID NO: 2, 4, 6, 8, 10, or 12. Preferably, that nucleic acid encodes at least: twelve consecutive residues from SEQ ID NO: 2, and further comprises a coding sequence of at least 17 nucleotides from SEQ ID NO: 1; twelve consecutive residues from SEQ ID NO: 4, and further comprises a coding sequence of at least 17 nucleotides from SEQ ID NO: 3; twelve consecutive residues from SEQ ID NO: 6, and further comprises a coding sequence of at least 17 nucleotides from SEQ ID NO: 5; twelve consecutive residues from SEQ ID NO: 8, and further comprises a coding sequence of at least 17 nucleotides from SEQ ID NO: 7; twelve consecutive residues from SEQ ID NO: 10, and further comprises a coding sequence of at least 17 nucleotides from SEQ ID NO: 9; or twelve consecutive residues from SEQ ID NO: 12, and further comprises a coding sequence of at least 17 nucleotides from SEQ ID NO: 11. In other preferred embodiments, the nucleic acid: exhibits at least about 80% identity to a natural cDNA encoding the segment; is in an expression vector; further comprises a promoter; further comprises an origin of replication; encodes a 3-fold or less substituted sequence from a natural sequence; is from a natural source; is detectably labeled; comprises synthetic nucleotide sequence; is less than 6 kb; is from a mammal; is attached to a solid substrate, including in a Southern or Northern blot; comprises a natural full length coding sequence; is in a cell; or is in a detection kit, which also comprises instructions for use or disposal of reagents therein.

Further embodiments include a nucleic acid which hybridizes under stringent wash conditions of 55° C and less than 150 mM salt to the nucleic acid; while preferred embodiments include those which exhibit at 5 least about 85% identity over a stretch of at least about 30 nucleotides to a primate sequence of SEQ ID NO: 1, 3, 5, 7, 9, or 11; or where the identity is at least 90%; or the stretch is at least 75 nucleotides; or where the identity is at least 95%; or the stretch is at least 100 10 nucleotides.

In other embodiments, the invention provides a binding compound comprising an antigen binding fragment from an antibody which binds to a mature TECK, MIP-3 α , MIP-3 β , DC CR, or M/DC CR protein. In various 15 embodiments, the binding compound is one wherein: the polypeptide is a mouse or human protein; the antibody is raised against a mature peptide sequence of SEQ ID NO: 2, 4, 6, 8, 10 or 12; the antibody is a monoclonal antibody; the binding compound is attached to a solid substrate; 20 the binding compound is in a sterile composition; the binding compound binds to a denatured antigen, including a detergent denatured antigen; the binding compound is detectably labeled; the binding compound is an Fv, Fab, or Fab2 fragment; the binding compound is conjugated to a 25 chemical moiety; the binding compound is in a detection kit which also comprises instructions for use or disposal of reagents therein.

The invention also provides a cell which makes the antibody.

30 The invention embraces methods of purifying a polypeptide using a binding compound to specifically separate said polypeptides from others; of generating an antigen-binding compound complex comprising the step of contacting a sample comprising the antigen to a sample 35 comprising a binding compound; or of modulating physiology or development of a cell expressing a receptor for a chemokine selected from TECK, MIP-3 α , or MIP-3 β ; the method comprising contacting the cell with a composition comprising an agonist or mutein of said

chemokine or an antibody antagonist of the chemokine. In certain embodiments of the method, the cell is a macrophage, lymphocyte, or eosinophil; or the physiology is a cellular calcium flux, a chemoattractant response, 5 cellular morphology modification responses, phosphoinositide lipid turnover, or an antiviral response. In other embodiments, the receptor is DC CR, the chemokine is MIP-3 α , the physiology is pulmonary physiology, or the cell is an eosinophil.

10

DESCRIPTION OF THE DRAWINGS

Figures 1 and 2A-2D show chemotactic properties of mTECK recombinant protein. Fig. 1 shows migration of mouse thymocytes to recombinant mTECK and effect of pertussis toxin. Chemotaxis assays were performed as described. Recombinant mouse lymphotactin was used as a positive control. Data are expressed as the mean of cell counts obtained from three separate experiments in duplicate \pm SEM. In one experiment, cells were pre 15 incubated 1 h with 10 ng/ml pertussis toxin (PTX) prior to the assay. Figs. 2A-2D show migration of other leukocyte subsets to recombinant mTECK. Mouse splenic dendritic cells (Fig. 2A) and mouse activated macrophages (Fig. 2B) were obtained. THP-1 human monocytic cells were 20 used without (Fig. 2C) or with (Fig. 2D) a 16 h activation with IFN- γ . Results are obtained as the mean of the 25 chemotactic index from three separate experiments per cell type in duplicate \pm SD. The number of cells migrating to medium alone was greater than 40 cells per 5 high power fields in each experiment. Recombinant MIP-1 α 30 was used as a positive control.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. General

The present invention provides DNA sequences 35 encoding various mammalian proteins which exhibit structural properties characteristic of a chemotactic cytokine, or chemokine. Other embodiments are directed to chemokine receptors. See, e.g., Lodi, et al. (1994) Science 263:1762-1767; Gronenborn and Clore (1991)

Protein Engineering 4:263-269; Miller and Kranger (1992) Proc. Nat'l Acad. Sci. USA 89:2950-2954; Matsushima and Oppenheim (1989) Cytokine 1:2-13; Stoeckle and Baker (1990) New Biol. 2:313-323; Oppenheim, et al. (1991) Ann. Rev. Immunol. 9:617-648; Schall (1991) Cytokine 3:165-183; and The Cytokine Handbook Academic Press, NY. Mouse and human embodiments are described herein.

Chemokines play an important role in immune and inflammatory responses by inducing migration and adhesion of leukocytes. These small secreted molecules are a growing superfamily of 8-14 kDa proteins characterized by a conserved four cysteine motif. See, e.g., Schall (1991) Cytokine 3:165-183; and Thomson (ed.) The Cytokine Handbook Academic Press, NY. Chemokines are secreted by activated leukocytes and act as a chemoattractant for a variety of cells which are involved in inflammation. Besides chemoattractant properties, chemokines have been shown to induce other biological responses, e.g., modulation of second messenger levels such as Ca^{++} ; inositol phosphate pool changes (see, e.g., Berridge (1993) Nature 361:315-325 or Billah and Anthes (1990) Biochem. J. 269:281-291); cellular morphology modification responses; phosphoinositide lipid turnover; possible antiviral responses; and others. Thus, the chemokines provided herein may, alone or in combination with other therapeutic reagents, have advantageous combination effects.

Moreover, there are reasons to suggest that chemokines may have effects on other cell types, e.g., attraction or activation of monocytes, dendritic cells, T cells, eosinophils, and/or perhaps on basophils and/or neutrophils. They may also have chemoattractive effects on various neural cells including, e.g., dorsal root ganglia neurons in the peripheral nervous system and/or central nervous system neurons.

Chemokine receptors are important in the signal transduction mechanisms mediated by the chemokines. They are useful markers for distinguishing cell populations,

and have been implicated as specific receptors for retroviral infections.

The chemokine superfamily was classically divided into two groups exhibiting characteristic structural motifs, the Cys-X-Cys (C-X-C) and Cys-Cys (C-C) families. These were distinguished on the basis of a single amino acid insertion between the NH-proximal pair of cysteine residues and sequence similarity. Typically, the C-X-C chemokines, i.e., IL-8 and MGSA/Gro- α act on neutrophils but not on monocytes, whereas the C-C chemokines, i.e., MIP-1 α and RANTES, are potent chemoattractants for monocytes and lymphocytes but not neutrophils. See, e.g., Miller, et al. (1992) *Crit. Rev. Immunol.* 12:17-46. A recently isolated chemokine, lymphotactin, does not belong to either group and may constitute a first member of a third chemokine family, the C family. Lymphotactin does not have a characteristic CC or CXC motif, and acts on lymphocytes but not neutrophils and monocytes. See, e.g., Kelner et al. (1994) *Science* 266:1395-1399. This chemokine defines a new C-C chemokine family. Even more recently, another chemokine exhibiting a CX3C motif has been identified, which establishes a fourth structural class.

The present invention provides additional chemokine reagents, e.g., nucleic acids, proteins and peptides, antibodies, etc., related to the newly discovered respective chemokines designated TECK; MIP-3 α , and MIP-3 β .

In other embodiments, the invention provides two genes encoding novel chemokine receptors, designated DC CR and M/DC CR. Their ligands have not yet specifically been identified. However, the receptors exhibit structural features typical of known chemokine receptors, e.g., 7 transmembrane spanning structures. They may exhibit properties of binding many different cytokines at varying specificities (shared or promiscuous binding specificity) or may exhibit high affinity for one (specific) or a subset (shared) of chemokines.

The described chemokines and receptors should be important for mediating various aspects of cellular, organ, tissue, or organismal physiology or development.

5 II. Purified chemokines

Mouse Thymus Expressed ChemoKine (TECK) nucleotide and amino acid sequences are shown in SEQ ID NO: 1 and 2, respectively. The signal sequence should run from 1 (Met) to about 23 (Ala), and removal of the signal 10 sequence should provide one natural mature sequence beginning at 24 (Gln). Human TECK nucleotide and amino acid sequences are shown in SEQ ID NO: 3 and 4, respectively. Signal sequence cleavage is probably between about Thr and Gln.

15 Nucleotide and amino acid sequences of another novel chemokine, from human, designated MIP-3 α are provided in SEQ ID NO: 5 and 6, respectively. Nucleotide and derived amino acid sequences of a third novel chemokine, from human, designated MIP-3 β are shown in SEQ ID NO: 7 and 8, 20 respectively. Signal sequence cleavage is about between Ser and Gly. Generic descriptions of physical properties of polypeptides, nucleic acids, and antibodies where directed to one embodiment clearly are generally applicable to other chemokines or receptors described 25 herein.

30 The nucleotide and amino acid sequences of a novel chemokine receptor found on dendritic cells (DC), from human, and designated DC CR, are provided in SEQ ID NO: 9 and 10, respectively. The nucleotide and amino acid sequences of another novel chemokine receptor found on macrophages and dendritic cells, from human, and designated M/DC CR, are provided in SEQ ID NO: 11 and 12. Alignment of M/DC CR with the previously 35 identified chemokine receptors CKR-1 through CKR-4 is shown in Table 1. Amino acid sequences for CKR-1 through CKR-4 are provided in SEQ ID NOS: 13-17.

The amino acid sequences for these new chemokines and receptors, provided amino to carboxy, are important in providing sequence information on the chemokine ligand

or receptor, allowing for distinguishing the protein from other proteins. Moreover, the sequences allow preparation of peptides to generate antibodies to recognize and distinguish such segments, and allow

5 preparation of oligonucleotide probes, both of which are strategies for isolation, e.g., cloning, of genes encoding such sequences, or related sequences, e.g., natural polymorphic or other variants. Similarities of the chemokines have been observed with other cytokines.

10 See, e.g., Bosenberg, et al. (1992) Cell 71:1157-1165; Huang, et. al. (1992) Molecular Biology of the Cell 3:349-362; and Pandiella, et al. (1992) J. Biol. Chem. 267:24028-24033. Likewise for the receptors.

15

Table 1: Alignment of M/DC CR with CKR-1 through CKR-4. The other chemokine receptors are SEQ ID NO: 13-17. An asterisk indicates fully conserved residue among all five receptors; a period represents conservative substitutions among all five receptors.

20

M/DC CR	MIYTRFLKGSLKMANYT LAPEDEYDV LIEGELESDEAEQCDKYDAQALS
C-C CKR-1	METPNNTTEDYDTTTEFDYGDATPCQKVNERAFG
C-C CKR-2	MLSTSRSR FIRNTNESGEEVTTFFF D YD YGAPCHKFDVKQIG
C-C CKR-3	MTTS LDTVETFGTTSYYDDVGLLCEKADTRALM
25 C-C CKR-4	MNPTDIADTLD ESIYSNYYLYESIPKPCTKEGIKAFG

* * .

30

M/DC CR	AQLVPSLCSAVFVIGVLDNLLVVLILVKYKGLKRVENIYLLNLAVSNLCF
C-C CKR-1	AQLLPPLYSLVFVIGLVGNILVVLVLVQYKRLKNMTSIYLLNLAI SDLLF
C-C CKR-2	AQLLPPLYSLVFIFGFVGNMLVVLILINCKKLKCLTDIYLLNLAI SDLLF
C-C CKR-3	AQFVPPPLYSLVFTVGLGNVVVVMILIKYRRRLIMTNIYLLNLAI SDLLF
35 C-C CKR-4	ELFLPPLYSLVVFVFGLLGN SVVVLVLFKYKRLRSMTDVYLLNLAI SDLLF

. * * * * *

40

M/DC CR	LLTLPFWAHAG-----GDPMCKI LIGLYFVGLYSETFFNCLLT VQRYL
C-C CKR-1	LFTLPFWIDYKLKD DWVFGDAMCKI LSGF YYTGLYSEIFFI ILLTIDRYL
C-C CKR-2	LITLPLWAH-SAANEWVFGNAMCKLFTGLYHIGYFGGIFFI ILLTIDRYL
C-C CKR-3	LVTLPFWIH YVRGHNWVFGHGMCKLLSGFYHTGLYSEIFFI ILLTIDRYL
45 C-C CKR-4	VFSLPFWGYYA-ADQWVFGGLCKMISWMYLVGFYSGIFFVMLMSIDRYL

. * * * *

45

M/DC CR	VFLHKGNFFSAR-RRVPCGIITSVLA WVTAILATLPEFV VYK PQMEDQKY
C-C CKR-1	AIVH--AVFALRARTVTFGVITSIIIWALAILASMPGLYFSKTQWEFTHH
C-C CKR-2	AIVH--AVFALKARTVTFGVITSIVTWGLAVLAALPEFIFYETEELFEET
C-C CKR-3	AIVH--AVFALRARTVTFGVITSIVTWGLAVLAALPEFIFYETEELFEET
50 C-C CKR-4	AIVH--AVFSLRARTLTYGVITSLATWSVAVFASLPGFLFSTCYTERNHT

. * * *

Table 1 (continued)

30

As used herein, the term "TECK" shall encompass, when used in a protein context, a protein having mature mouse or human amino acid sequences, as shown in SEQ ID NO: 2 or SEQ ID NO: 4. The invention also embraces a polypeptide comprising a significant fragment of such protein. It also refers to a polypeptide that is a species counterpart, e.g., which exhibits similar biological function, and is more homologous in natural encoding sequence than other genes from that species. Typically, such chemokine will also interact with its specific binding components, e.g., receptor. These binding components, e.g., antibodies, typically bind to the chemokine with high affinity, e.g., at least about 100 nM, usually better than about 30 nM, preferably better than about 10 nM, and more preferably at better than about 3 nM. Homologous proteins would be found in mammalian species other than mouse, e.g., rats, dogs, cats, and primates. Non-mammalian species should also possess structurally or functionally related genes and proteins.

The term "polypeptide" as used herein includes a significant fragment or segment, and encompasses a stretch of amino acid residues of at least about 8 amino acids, generally at least 10 amino acids, more generally 5 at least 12 amino acids, often at least 14 amino acids, more often at least 16 amino acids, typically at least 18 amino acids, more typically at least 20 amino acids, usually at least 22 amino acids, more usually at least 24 amino acids, preferably at least 26 amino acids, more 10 preferably at least 28 amino acids, and, in particularly preferred embodiments, at least about 30 or more amino acids, e.g., about 35, 40, 45, 50, 60, 75, 80, 100, 120, etc. Similar proteins will likely comprise a plurality of such segments. Such fragments may have ends that 15 begin and/or end at virtually all positions, e.g., beginning at residues 1, 2, 3, etc., and ending at, e.g., 69, 68, 67, 66, etc., in all combinations. Particularly interesting peptides have ends corresponding to structural domain boundaries.

20 The term "binding composition" refers to molecules that bind with specificity to the respective chemokine or receptor, e.g., in a ligand-receptor type fashion or an antibody-antigen interaction. These compositions may be compounds, e.g., proteins, which specifically associate 25 with the chemokine or receptor, including natural physiologically relevant protein-protein interactions, either covalent or non-covalent. The binding composition may be a polymer, or another chemical reagent. No implication as to whether the chemokine presents a 30 concave or convex shape in its ligand-receptor interaction is represented, other than the interaction exhibit similar specificity, e.g., specific affinity. A functional analog may be a ligand with structural modifications, or may be a wholly unrelated molecule, 35 e.g., which has a molecular shape that interacts with the appropriate ligand binding determinants. The ligands may serve as agonists or antagonists of the receptor, see, e.g., Goodman, et al. (eds.) (1990) Goodman & Gilman's:

The Pharmacological Bases of Therapeutics (8th ed.),
Pergamon Press.

Substantially pure means that the protein is free from other contaminating proteins, nucleic acids, and/or other biologicals typically derived from the original source organism. Purity may be assayed by standard methods, and will ordinarily be at least about 40% pure, more ordinarily at least about 50% pure, generally at least about 60% pure, more generally at least about 70% pure, often at least about 75% pure, more often at least about 80% pure, typically at least about 85% pure, more typically at least about 90% pure, preferably at least about 95% pure, more preferably at least about 98% pure, and in most preferred embodiments, at least 99% pure.

Analyses will typically be by weight, but may be by molar amounts.

Solubility of a polypeptide or fragment depends upon the environment and the polypeptide. Many parameters affect polypeptide solubility, including temperature, electrolyte environment, size and molecular characteristics of the polypeptide, and nature of the solvent. Typically, the temperature at which the polypeptide is used ranges from about 4° C to about 65° C. Usually the temperature at use is greater than about 18° C and more usually greater than about 22° C. For diagnostic purposes, the temperature will usually be about room temperature or warmer, but less than the denaturation temperature of components in the assay. For therapeutic purposes, the temperature will usually be body temperature, typically about 37° C for humans, though under certain situations the temperature may be raised or lowered in situ or in vitro.

The electrolytes will usually approximate in situ physiological conditions, but may be modified to higher or lower ionic strength where advantageous. The actual ions may be modified, e.g., to conform to standard buffers used in physiological or analytical contexts.

The size and structure of the polypeptide should generally be in a substantially stable state, and usually

not in a denatured state. The polypeptide may be associated with other polypeptides in a quaternary structure, e.g., to confer solubility, or associated with lipids or detergents in a manner that approximates natural lipid bilayer interactions.

5 The solvent will usually be a biologically compatible buffer, of a type used for preservation of biological activities, and will usually approximate a physiological solvent. Usually the solvent will have a 10 neutral pH, typically between about 5 and 10, and preferably about 7.5. On some occasions, a detergent will be added, typically a mild non-denaturing one, e.g., CHS or CHAPS, or a low enough concentration as to avoid significant disruption of structural or physiological 15 properties of the protein.

Solubility is reflected by sedimentation measured in Svedberg units, which are a measure of the sedimentation velocity of a molecule under particular conditions. The determination of the sedimentation velocity was 20 classically performed in an analytical ultracentrifuge, but is typically now performed in a standard ultracentrifuge. See, Freifelder (1982) Physical Biochemistry (2d ed.), W.H. Freeman; and Cantor and Schimmel (1980) Biophysical Chemistry, parts 1-3, W.H. 25 Freeman & Co., San Francisco. As a crude determination, a sample containing a putatively soluble polypeptide is spun in a standard full sized ultracentrifuge at about 50K rpm for about 10 minutes, and soluble molecules will remain in the supernatant. A soluble particle or 30 polypeptide will typically be less than about 30S, more typically less than about 15S, usually less than about 10S, more usually less than about 6S, and, in particular embodiments, preferably less than about 4S, and more preferably less than about 3S.

III. Physical Variants

This invention also encompasses proteins or peptides having substantial amino acid sequence homology with the 5 amino acid sequence of each respective chemokine or receptor. The variants include species or polymorphic variants.

Amino acid sequence homology, or sequence identity, is determined by optimizing residue matches, if 10 necessary, by introducing gaps as required. This changes when considering conservative substitutions as matches.

15 Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

Homologous amino acid sequences are typically intended to include natural allelic and interspecies variations in each respective protein sequence. Typical homologous 20 proteins or peptides will have from 25-100% homology (if gaps can be introduced), to 50-100% homology (if conservative substitutions are included) with the amino acid sequence of the appropriate chemokine or receptor. Homology measures will be at least about 35%, generally 25 at least 40%, more generally at least 45%, often at least 50%, more often at least 55%, typically at least 60%, more typically at least 65%, usually at least 70%, more usually at least 75%, preferably at least 80%, and more preferably at least 80%, and in particularly preferred 30 embodiments, at least 85% or more. See also Needleham, et al. (1970) J. Mol. Biol. 48:443-453; Sankoff, et al. (1983) Chapter One in Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison Addison-Wesley, Reading, MA; and software 35 packages from IntelliGenetics, Mountain View, CA; and the University of Wisconsin Genetics Computer Group, Madison, WI.

Each of the isolated chemokine or receptor DNAs can be readily modified by nucleotide substitutions,

nucleotide deletions, nucleotide insertions, and inversions of nucleotide stretches. These modifications result in novel DNA sequences that encode these antigens, their derivatives, or proteins having similar

5 physiological, immunogenic, or antigenic activity. These modified sequences can be used to produce mutant antigens or to enhance expression. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. Such mutant

10 chemokine or receptor derivatives include predetermined or site-specific mutations of the respective protein or its fragments. "Mutant chemokine" encompasses a polypeptide otherwise falling within the homology definition of the chemokine as set forth above, but

15 having an amino acid sequence that differs from that of the chemokine as found in nature, whether by way of deletion, substitution, or insertion. These include substitution levels from none, one, two, three, etc. In particular, "site specific mutant chemokine" generally

20 includes proteins having significant homology with a ligand having sequences of SEQ ID NO: 2, 4, 6 or 8, and as sharing various biological activities, e.g., antigenic or immunogenic, with those sequences, and in preferred embodiments contain most of the disclosed sequences.

25 Similar concepts apply to the different chemokine protein embodiments, particularly those found in various warm blooded animals, e.g., mammals and birds. As stated before, it is emphasized that descriptions are generally meant to encompass the various chemokine proteins, not

30 limited to the mouse or human embodiments specifically discussed. Similar concepts apply to the receptor embodiments.

Although site specific mutation sites are often predetermined, mutants need not be site specific.

35 Chemokine mutagenesis can be conducted by making amino acid insertions or deletions. Substitutions, deletions, insertions, or combinations may be generated to arrive at a final construct. Insertions include amino- or carboxy-terminal fusions. Random mutagenesis can be conducted at

a target codon and the expressed mutants can then be screened for the desired activity. Methods for making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art, e.g., 5 by M13 primer mutagenesis or polymerase chain reaction (PCR) techniques. See also Sambrook, et al. (1989) and Ausubel, et al. (1987 and Supplements).

The mutations in the DNA normally should not place coding sequences out of reading frames and preferably 10 will not create complementary regions that could hybridize to produce secondary mRNA structure such as loops or hairpins.

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using 15 segments from these proteins. A heterologous fusion protein is a fusion of proteins or segments that are naturally not normally fused in the same manner. Thus, the fusion product of an immunoglobulin with a chemokine or receptor polypeptide is a continuous protein molecule 20 having sequences fused in a typical peptide linkage, typically made as a single translation product and exhibiting properties derived from each source peptide. A similar chimeric concept applies to heterologous nucleic acid sequences.

25 In addition, new constructs may be made from combining similar functional domains from other proteins. For example, ligand-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g., Cunningham, et al. (1989) Science 30 243:1330-1336; and O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992. Thus, new chimeric polypeptides exhibiting new combinations of specificities will result from the functional linkage of ligand-binding specificities and other functional domains.

35 The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the

strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence, e.g., PCR techniques.

5 IV. Functional Variants

The blocking of physiological response to various embodiments of these chemokines may result from the inhibition of binding of the ligand to its receptor, likely through competitive inhibition. Thus, in vitro 10 assays of the present invention will often use isolated protein, membranes from cells expressing a recombinant membrane associated chemokine, soluble fragments comprising receptor binding segments of these ligands, or fragments attached to solid phase substrates. These 15 assays will also allow for the diagnostic determination of the effects of either binding segment mutations and modifications, or ligand mutations and modifications, e.g., ligand analogs.

This invention also contemplates the use of 20 competitive drug screening assays, e.g., where neutralizing antibodies to antigen or receptor fragments compete with a test compound for binding to the protein. In this manner, the antibodies can be used to detect the presence of polypeptides that share one or more antigenic 25 binding sites of the ligand and can also be used to occupy binding sites on the protein that might otherwise interact with a receptor.

Additionally, neutralizing antibodies against a specific chemokine embodiment and soluble fragments of 30 the chemokine that contain a high affinity receptor binding site, can be used to inhibit chemokine activity in tissues, e.g., tissues experiencing abnormal physiology.

"Derivatives" of chemokine antigens include amino 35 acid sequence mutants, glycosylation variants, and covalent or aggregate conjugates with other chemical moieties. Covalent derivatives can be prepared by linkage of functionalities to groups which are found in chemokine amino acid side chains or at the N- or C-

termini, by means which are well known in the art. These derivatives can include, without limitation, aliphatic esters or amides of the carboxyl terminus, or of residues containing carboxyl side chains, O-acyl derivatives of 5 hydroxyl group-containing residues, and N-acyl derivatives of the amino terminal amino acid or amino-group containing residues, e.g., lysine or arginine. Acyl groups are selected from the group of alkyl-moieties including C3 to C18 normal alkyl, thereby forming 10 alkanoyl aroyl species. Covalent attachment to carrier proteins may be important when immunogenic moieties are haptens.

In particular, glycosylation alterations are included, e.g., made by modifying the glycosylation 15 patterns of a polypeptide during its synthesis and processing, or in further processing steps. Particularly preferred means for accomplishing this are by exposing the polypeptide to glycosylating enzymes derived from cells which normally provide such processing, e.g., 20 mammalian glycosylation enzymes. Deglycosylation enzymes are also contemplated. Also embraced are versions of the same primary amino acid sequence which have other minor modifications, including phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or 25 phosphothreonine.

A major group of derivatives are covalent conjugates of the respective chemokine or receptor or fragments thereof with other proteins or polypeptides. These derivatives can be synthesized in recombinant culture 30 such as N- or C-terminal fusions or by the use of agents known in the art for their usefulness in cross-linking proteins through reactive side groups. Preferred chemokine derivatization sites with cross-linking agents are at free amino groups, carbohydrate moieties, and 35 cysteine residues.

Fusion polypeptides between these chemokines and other homologous or heterologous proteins, e.g., other chemokines, are also provided. Many growth factors and cytokines are homodimeric entities, and a repeat

construct may have various advantages, including lessened susceptibility to proteolytic cleavage. Moreover, many receptors require dimerization to transduce a signal, and various dimeric ligands or domain repeats can be

5 desirable. Homologous polypeptides may be fusions between different surface markers, resulting in, e.g., a hybrid protein exhibiting receptor binding specificity. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities

10 of the derivative proteins. Typical examples are fusions of a reporter polypeptide, e.g., luciferase, with a segment or domain of a ligand, e.g., a receptor-binding segment, so that the presence or location of the fused ligand may be easily determined. See, e.g., Dull, et

15 al., U.S. Patent No. 4,859,609. Other gene fusion partners include bacterial β -galactosidase, trpE, Protein A, β -lactamase, alpha amylase, alcohol dehydrogenase, a FLAG fusion, and yeast alpha mating factor. See, e.g., Godowski, et al. (1988) Science 241:812-816.

20 The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the

25 strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Such polypeptides may also have amino acid residues that have been chemically modified by phosphorylation, sulfonation, biotinylation, or the addition or removal of other moieties, particularly those which have molecular shapes similar to phosphate groups. In some embodiments, the modifications will be useful labeling reagents, or serve as purification targets, e.g., affinity tags as

35 FLAG.

Fusion proteins will typically be made by either recombinant nucleic acid methods or by synthetic polypeptide methods. Techniques for nucleic acid manipulation and expression are described generally, for

example, in Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.), Vols. 1-3, Cold Spring Harbor Laboratory. Techniques for synthesis of polypeptides are described, for example, in Merrifield (1963) J. Amer. Chem. Soc. 85:2149-2156; Merrifield (1986) Science 232: 341-347; and Atherton, et al. (1989) Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, Oxford; and chemical ligation, e.g., Dawson, et al. (1994) Science 266:776-779, a method of linking long synthetic peptides by a peptide bond.

This invention also contemplates the use of derivatives of these chemokines or receptors other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties. These derivatives generally fall into the three classes: (1) salts, (2) side chain and terminal residue covalent modifications, and (3) adsorption complexes, for example with cell membranes. Such covalent or aggregative derivatives are useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of ligands or other binding ligands. For example, a chemokine antigen can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated Sepharose, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of anti-chemokine antibodies or its receptor. These chemokines can also be labeled with a detectable group, for example radioiodinated by the chloramine T procedure, covalently bound to rare earth chelates, or conjugated to a fluorescent moiety for use in diagnostic assays. Purification of chemokine may be effected by immobilized antibodies or receptor.

Other modifications may be introduced with the goal of modifying the therapeutic pharmacokinetics or pharmacodynamics of a target chemokine. For example, certain means to minimize the size of the entity may

improve its pharmacoaccessibility; other means to maximize size may affect pharmacodynamics.

A solubilized chemokine or appropriate fragment of this invention can be used as an immunogen for the 5 production of antisera or antibodies specific for the ligand or fragments thereof. The purified chemokines can be used to screen monoclonal antibodies or chemokine-binding fragments prepared by immunization with various forms of impure preparations containing the protein. In 10 particular, antibody equivalents include antigen binding fragments of natural antibodies, e.g., Fv, Fab, or F(ab)2. Purified chemokines can also be used as a reagent to detect antibodies generated in response to the presence of elevated levels of the protein or cell 15 fragments containing the protein, both of which may be diagnostic of an abnormal or specific physiological or disease condition. Additionally, chemokine protein fragments, or their concatenates, may also serve as immunogens to produce antibodies of the present 20 invention, as described immediately below. For example, this invention contemplates antibodies raised against amino acid sequences shown in SEQ ID NO: 2, 4, 6 or 8, or proteins containing them. In particular, this invention contemplates antibodies having binding affinity 25 to or being raised against specific fragments, e.g., those which are predicted to lie on the outside surfaces of protein tertiary structure. Similar concepts apply to antibodies specific for receptors of the invention.

The present invention contemplates the isolation of 30 additional closely related species variants. Southern and Northern blot analysis should establish that similar genetic entities exist in other mammals, and establish the stringency of hybridization conditions to isolate such. It is likely that these chemokines and receptors 35 are widespread in species variants, e.g., rodents, lagomorphs, carnivores, artiodactyla, perissodactyla, and primates.

The invention also provides means to isolate a group of related chemokines displaying both distinctness and

similarities in structure, expression, and function. Elucidation of many of the physiological effects of the proteins will be greatly accelerated by the isolation and characterization of distinct species variants of the 5 ligands. In particular, the present invention provides useful probes for identifying additional homologous genetic entities in different species.

The isolated genes will allow transformation of cells lacking expression of a corresponding chemokine, 10 e.g., either species types or cells that lack corresponding ligands and exhibit negative background activity. Expression of transformed genes will allow isolation of antigenically pure cell lines, with defined or single species variants. This approach will allow for 15 more sensitive detection and discrimination of the physiological effects of chemokine receptor proteins. Subcellular fragments, e.g., cytoplasts or membrane fragments, can be isolated and used.

Dissection of critical structural elements which 20 effect the various differentiation functions provided by ligands is possible using standard techniques of modern molecular biology, particularly in comparing members of the related class. See, e.g., the homolog-scanning mutagenesis technique described in Cunningham, et al. 25 (1989) Science 243:1339-1336; and approaches used in O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992; and Lechleiter, et al. (1990) EMBO J. 9:4381-4390.

In addition, receptor binding segments can be 30 substituted between species variants to determine what structural features are important in both receptor binding affinity and specificity, as well as signal transduction. An array of different chemokine variants will be used to screen for ligands exhibiting combined properties of interaction with different receptor species 35 variants.

Intracellular functions would probably involve segments of the receptor that are normally accessible to the cytosol. However, ligand internalization may occur under certain circumstances, and interaction between

intracellular components and "extracellular" segments may occur. The specific segments of interaction of a particular chemokine with other intracellular components may be identified by mutagenesis or direct biochemical

5 means, e.g., cross-linking or affinity methods.

Structural analysis by crystallographic or other physical methods will also be applicable. Further investigation of the mechanism of signal transduction will include study of associated components that may be isolatable by 10 affinity methods or by genetic means, e.g., complementation analysis of mutants.

Further study of the expression and control of the various chemokines will be pursued. The controlling elements associated with the proteins may exhibit 15 differential developmental, tissue specific, or other expression patterns. Upstream or downstream genetic regions, e.g., control elements, are of interest. Differential splicing of message may lead to membrane bound forms, soluble forms, and modified versions of 20 ligand.

Structural studies of the proteins will lead to design of new ligands, particularly analogs exhibiting agonist or antagonist properties on the receptor. This 25 can be combined with previously described screening methods to isolate ligands exhibiting desired spectra of activities.

Expression in other cell types will often result in glycosylation differences in a particular chemokine. Various species variants may exhibit distinct functions 30 based upon structural differences other than amino acid sequence. Differential modifications may be responsible for differential function, and elucidation of the effects is now made possible.

Thus, the present invention provides important 35 reagents related to a physiological chemokine-binding protein interaction. Although the foregoing description has focused primarily upon the mouse and human embodiments of the chemokines specifically described, those of skill in the art will immediately recognize that

the invention provides other species counterparts, e.g., rat and other mammalian species or allelic or polymorphic variants.

5 V. Antibodies

Antibodies can be raised to these chemokines, including species or polymorphic variants, and fragments thereof, both in their naturally occurring forms and in their recombinant forms. Additionally, antibodies can be 10 raised to chemokines in either their active forms or in their inactive forms. Anti-idiotypic antibodies are also contemplated.

Antibodies, including binding fragments and single chain versions, against predetermined fragments of the 15 ligands can be raised by immunization of animals with concatemers or conjugates of the fragments with immunogenic proteins. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or 20 defective chemokines, or screened for agonistic or antagonistic activity, e.g., mediated through a receptor for a chemokine. These monoclonal antibodies will usually bind with at least a K_D of about 1 mM, more usually at least about 300 μ M, typically at least about 25 10 μ M, more typically at least about 30 μ M, preferably at least about 10 μ M, and more preferably at least about 3 μ M or better.

The antibodies, including antigen binding fragments, of this invention can have significant diagnostic or 30 therapeutic value. They can be potent antagonists that bind to ligand and inhibit binding to receptor or inhibit the ability of a ligand to elicit a biological response. They also can be useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides so that 35 when the antibody binds to ligand, a cell expressing it, e.g., on its surface via receptor, is killed. Further, these antibodies can be conjugated to drugs or other therapeutic agents, either directly or indirectly by means of a linker, and may effect drug targeting.

Antibodies to receptors may be more easily used to block ligand binding and signal transduction.

The antibodies of this invention can also be useful in diagnostic or reagent purification applications. As 5 capture or non-neutralizing antibodies, they can be screened for ability to bind to the chemokines without inhibiting receptor binding. As neutralizing antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying chemokine 10 or, indirectly, receptors, e.g., in immunoassays. They may be used as purification reagents in immunoaffinity columns or as immunohistochemistry reagents.

Ligand fragments may be concatenated or joined to other materials, particularly polypeptides, as fused or 15 covalently joined polypeptides to be used as immunogens. Short peptides will preferably be made as repeat structures to increase size. A ligand and its fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine 20 serum albumin, tetanus toxoid, etc. See Microbiology, Hoeber Medical Division, Harper and Row, 1969; Landsteiner (1962) Specificity of Serological Reactions, Dover Publications, New York, and Williams, et al. (1967) Methods in Immunology and Immunochemistry, Vol. 1, 25 Academic Press, New York, for descriptions of methods of preparing polyclonal antisera. A typical method involves hyperimmunization of an animal with an antigen. The blood of the animal is then collected shortly after the repeated immunizations and the gamma globulin fraction is 30 isolated.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may 35 be found in, e.g., Stites, et al. (eds.) Basic and Clinical Immunology (4th ed.), Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press; Goding (1986) Monoclonal Antibodies:

Principles and Practice (2d ed.) Academic Press, New York; and particularly in Kohler and Milstein (1975) in Nature 256:495-497, which discusses one method of generating monoclonal antibodies. Summarized briefly, 5 this method involves injecting an animal with an immunogen. The animal is then sacrificed and cells taken, e.g., from its spleen, which are then fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing in vitro. The 10 population of hybridomas is then screened to isolate individual clones, each of which secrete a single antibody species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immune 15 animal generated in response to a specific site recognized on the immunogenic substance. Large amounts of antibody may be derived from ascites fluid from an animal.

Other suitable techniques involve in vitro exposure 20 of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors. See, Huse, et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," Science 25 246:1275-1281; and Ward, et al. (1989) Nature 341:544- 546. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by 30 joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, 35 enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents, teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also,

recombinant immunoglobulins may be produced, see Cabilly, U.S. Patent No. 4,816,567; and Queen et al. (1989) Proc. Nat'l. Acad. Sci. 86:10029-10033.

The antibodies of this invention can also be used
5 for affinity chromatography in isolating the protein. Columns can be prepared where the antibodies are linked to a solid support, e.g., particles, such as agarose, Sephadex, or the like, where a cell lysate may be passed through the column, the column washed, followed by
10 increasing concentrations of a mild denaturant, whereby the purified chemokine protein will be released.

The antibodies may also be used to screen expression libraries for particular expression products. Usually the antibodies used in such a procedure will be labeled
15 with a moiety allowing easy detection of presence of antigen by antibody binding.

Antibodies raised against these chemokine will also be useful to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various
20 immunological conditions related to expression of the respective antigens.

VI. Nucleic Acids

The described peptide sequences and the related
25 reagents are useful in isolating a DNA clone encoding these chemokines, e.g., from a natural source. Typically, it will be useful in isolating a gene from another individual, and similar procedures will be applied to isolate genes from other species, e.g., warm
30 blooded animals, such as birds and mammals. Cross hybridization will allow isolation of ligand from other species. A number of different approaches should be available to successfully isolate a suitable nucleic acid clone. Similar concepts apply to the receptor
35 embodiments.

The purified protein or defined peptides are useful for generating antibodies by standard methods, as described above. Synthetic peptides or purified protein can be presented to an immune system to generate

monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. Alternatively, a chemokine receptor can be used as a specific binding reagent, and advantage can be taken of its specificity of binding, much like an antibody would be used. However, chemokine receptors are typically 7 transmembrane proteins, which could be sensitive to appropriate interaction with lipid or membrane. The signal transduction typically is mediated through a G-protein.

For example, the specific binding composition could be used for screening of an expression library made from a cell line which expresses a particular chemokine. The screening can be standard staining of surface expressed ligand, or by panning. Screening of intracellular expression can also be performed by various staining or immunofluorescence procedures. The binding compositions could be used to affinity purify or sort out cells expressing the ligand.

The peptide segments can also be used to predict appropriate oligonucleotides to screen a library, e.g., to isolate species variants. The genetic code can be used to select appropriate oligonucleotides useful as probes for screening. In combination with polymerase chain reaction (PCR) techniques, synthetic oligonucleotides will be useful in selecting correct clones from a library. Complementary sequences will also be used as probes or primers. Based upon identification of the likely amino terminus, the third peptide should be particularly useful, e.g., coupled with anchored vector or poly-A complementary PCR techniques or with complementary DNA of other peptides.

This invention contemplates use of isolated DNA or fragments to encode a biologically active corresponding chemokine polypeptide. In addition, this invention covers isolated or recombinant DNA which encodes a biologically active protein or polypeptide which is capable of hybridizing under appropriate conditions with

the DNA sequences described herein. Said biologically active protein or polypeptide can be an intact ligand, or fragment, and have an amino acid sequence as disclosed in SEQ ID NOS: 1, 3, 5 and 7. Further, this invention 5 covers the use of isolated or recombinant DNA, or fragments thereof, which encode proteins which are homologous to a chemokine or which was isolated using cDNA encoding a chemokine as a probe. The isolated DNA can have the respective regulatory sequences in the 5' 10 and 3' flanks, e.g., promoters, enhancers, poly-A addition signals, and others.

An "isolated" nucleic acid is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other components which naturally accompany 15 a native sequence, e.g., ribosomes, polymerases, and flanking genomic sequences from the originating species. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and 20 chemically synthesized analogs or analogs biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule.

An isolated nucleic acid will generally be a homogeneous composition of molecules, but will, in some 25 embodiments, contain minor heterogeneity. This heterogeneity is typically found at the polymer ends or portions not critical to a desired biological function or activity.

A "recombinant" nucleic acid is defined either by 30 its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence, typically selection or production. 35 Alternatively, it can be a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants. Thus, for example, products made by

transforming cells with any unnaturally occurring vector is encompassed, as are nucleic acids comprising sequence derived using any synthetic oligonucleotide process. Such is often done to replace a codon with a redundant 5 codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a desired 10 combination of functions not found in the commonly available natural forms. Restriction enzyme recognition sites are often the target of such artificial manipulations, but other site specific targets, e.g., promoters, DNA replication sites, regulation sequences, 15 control sequences, or other useful features may be incorporated by design. A similar concept is intended for a recombinant, e.g., fusion, polypeptide. Specifically included are synthetic nucleic acids which, by genetic code redundancy, encode polypeptides similar 20 to fragments of these antigens, and fusions of sequences from various different species variants.

A significant "fragment" in a nucleic acid context is a contiguous segment of at least about 17 nucleotides, generally at least about 20 nucleotides, more generally 25 at least about 23 nucleotides, ordinarily at least about 26 nucleotides, more ordinarily at least about 29 nucleotides, often at least about 32 nucleotides, more often at least about 35 nucleotides, typically at least about 38 nucleotides, more typically at least about 41 30 nucleotides, usually at least about 44 nucleotides, more usually at least about 47 nucleotides, preferably at least about 50 nucleotides, more preferably at least about 53 nucleotides, and in particularly preferred embodiments will be at least about 56 or more 35 nucleotides, e.g., 60, 65, 75, 85, 100, 120, 150, 200, 250, 300, 400, etc. Such fragments may have ends which begin and/or end at virtually all positions, e.g., beginning at nucleotides 1, 2, 3, etc., and ending at, e.g., 300, 299, 298, 287, etc., in all combinations.

Particularly interesting polynucleotides have ends corresponding to structural domain boundaries.

A DNA which codes for a particular chemokine protein or peptide will be very useful to identify genes, mRNA,

5 and cDNA species which code for related or homologous ligands, as well as DNAs which code for homologous proteins from different species. There are likely homologs in other species, including primates. Various chemokine proteins should be homologous and are 10 encompassed herein. However, even proteins that have a more distant evolutionary relationship to the ligand can readily be isolated under appropriate conditions using these sequences if they are sufficiently homologous. Primate chemokines are of particular interest.

15 This invention further covers recombinant DNA molecules and fragments having a DNA sequence identical to or highly homologous to the isolated DNAs set forth herein. In particular, the sequences will often be operably linked to DNA segments which control

20 transcription, translation, and DNA replication.

Alternatively, recombinant clones derived from the genomic sequences, e.g., containing introns, will be useful for transgenic studies, including, e.g., transgenic cells and organisms, and for gene therapy.

25 See, e.g., Goodnow (1992) "Transgenic Animals" in Roitt (ed.) Encyclopedia of Immunology Academic Press, San Diego, pp. 1502-1504; Travis (1992) Science 256:1392-1394; Kuhn, et al. (1991) Science 254:707-710; Capecchi (1989) Science 244:1288; Robertson (1987) (ed.)

30 Teratocarcinomas and Embryonic Stem Cells: A Practical Approach IRL Press, Oxford; and Rosenberg (1992) J. Clinical Oncology 10:180-199.

Homologous nucleic acid sequences, when compared, exhibit significant similarity, or identity. The

35 standards for homology in nucleic acids are either measures for homology generally used in the art by sequence comparison or based upon hybridization conditions. The hybridization conditions are described in greater detail below.

Substantial homology in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when optimally aligned, with appropriate nucleotide

5 insertions or deletions, in at least about 50% of the nucleotides, generally at least about 56%, more generally at least about 59%, ordinarily at least about 62%, more ordinarily at least about 65%, often at least about 68%, more often at least about 71%, typically at least about

10 74%, more typically at least about 77%, usually at least about 80%, more usually at least about 85%, preferably at least about 90%, more preferably at least about 95 to 98% or more, and in particular embodiments, as high as about 99% or more of the nucleotides. Alternatively,

15 substantial homology exists when the segments will hybridize under selective hybridization conditions, to a strand, or its complement, typically using a sequence derived from those set forth in SEQ ID NOS: 1, 3, 4, 7, 9 or 11. Typically, selective hybridization will occur

20 when there is at least about 55% homology over a stretch of at least about 30 nucleotides, preferably at least about 65% over a stretch of at least about 25 nucleotides, more preferably at least about 75%, and most preferably at least about 90% over about 20 nucleotides.

25 See, Kanehisa (1984) Nuc. Acids Res. 12:203-213. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, usually at least about 20 nucleotides, more usually at least about

30 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 40 nucleotides, preferably at least about 50 nucleotides, and more preferably at least about 75 to 100 or more nucleotides.

Stringent conditions, in referring to homology in

35 the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters, typically those controlled in hybridization reactions. Stringent temperature conditions will usually include temperatures in excess of

about 30° C, more usually in excess of about 37° C, typically in excess of about 45° C, more typically in excess of about 55° C, preferably in excess of about 65° C, and more preferably in excess of about 70° C.

5 Stringent salt conditions will ordinarily be less than about 1000 mM, usually less than about 500 mM, more usually less than about 400 mM, typically less than about 300 mM, preferably less than about 200 mM, and more preferably less than about 150 mM. However, the
10 combination of parameters is much more important than the measure of any single parameter. See, e.g., Wetmur and Davidson (1968) J. Mol. Biol. 31:349-370.

Corresponding chemokines from other mammalian species can be cloned and isolated by cross-species
15 hybridization of closely related species. Alternatively, sequences from a data base may be recognized as having similarity. Homology may be relatively low between distantly related species, and thus hybridization of relatively closely related species is advisable.

20 Alternatively, preparation of an antibody preparation which exhibits less species specificity may be useful in expression cloning approaches. PCR approaches using segments of conserved sequences will also be used.

25 VII. Making chemokine; Mimetics

DNA which encodes each respective chemokine or fragments thereof can be obtained by chemical synthesis, screening cDNA libraries, or by screening genomic libraries prepared from a wide variety of cell lines or
30 tissue samples.

This DNA can be expressed in a wide variety of host cells for the synthesis of a full-length ligand or fragments which can in turn, for example, be used to generate polyclonal or monoclonal antibodies; for binding
35 studies; for construction and expression of modified molecules; and for structure/function studies. Each antigen or its fragments can be expressed in host cells that are transformed or transfected with appropriate expression vectors. These molecules can be substantially

purified to be free of protein or cellular contaminants, other than those derived from the recombinant host, and therefore are particularly useful in pharmaceutical compositions when combined with a pharmaceutically acceptable carrier and/or diluent. The antigen, or portions thereof, may be expressed as fusions with other proteins.

5 Expression vectors are typically self-replicating DNA or RNA constructs containing the desired antigen gene 10 or its fragments, usually operably linked to suitable genetic control elements that are recognized in a suitable host cell. These control elements are capable of effecting expression within a suitable host. The specific type of control elements necessary to effect 15 expression will depend upon the eventual host cell used. Generally, the genetic control elements can include a prokaryotic promoter system or a eukaryotic promoter expression control system, and typically include a transcriptional promoter, an optional operator to control 20 the onset of transcription, transcription enhancers to elevate the level of mRNA expression, a sequence that encodes a suitable ribosome binding site, and sequences that terminate transcription and translation. Expression vectors also usually contain an origin of replication 25 that allows the vector to replicate independently of the host cell.

The vectors of this invention contain DNA which encodes embodiments of a chemokine, receptor, or a fragment thereof, typically encoding a biologically active polypeptide. The DNA can be under the control of 30 a viral promoter and can encode a selection marker. This invention further contemplates use of such expression vectors which are capable of expressing eukaryotic cDNA coding for each chemokine in a prokaryotic or eukaryotic 35 host, where the vector is compatible with the host and where the eukaryotic cDNA coding for the ligand is inserted into the vector such that growth of the host containing the vector expresses the cDNA in question. Usually, expression vectors are designed for stable

replication in their host cells or for amplification to greatly increase the total number of copies of the desirable gene per cell. It is not always necessary to require that an expression vector replicate in a host cell, e.g., it is possible to effect transient expression of the ligand or its fragments in various hosts using vectors that do not contain a replication origin that is recognized by the host cell. It is also possible to use vectors that cause integration of a chemokine gene or its fragments into the host DNA by recombination, or to integrate a promoter which controls expression of an endogenous gene.

Vectors, as used herein, comprise plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. Expression vectors are specialized vectors which contain genetic control elements that effect expression of operably linked genes. Plasmids are the most commonly used form of vector but all other forms of vectors which serve an equivalent function and which are, or become, known in the art are suitable for use herein. See, e.g., Pouwels, et al. (1985 and Supplements) Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., and Rodriguez, et al. (1988) (eds.) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworth, Boston, MA.

Transformed cells include cells, preferably mammalian, that have been transformed or transfected with a chemokine gene containing vector constructed using recombinant DNA techniques. Transformed host cells usually express the ligand or its fragments, but for purposes of cloning, amplifying, and manipulating its DNA, do not need to express the protein. This invention further contemplates culturing transformed cells in a nutrient medium, thus permitting the protein to accumulate in the culture. The protein can be recovered, either from the culture or from the culture medium.

For purposes of this invention, DNA sequences are operably linked when they are functionally related to

each other. For example, DNA for a presequence or secretory leader is operably linked to a polypeptide if it is expressed as a preprotein or participates in directing the polypeptide to the cell membrane or in secretion of the polypeptide. A promoter is operably linked to a coding sequence if it controls the transcription of the polypeptide; a ribosome binding site is operably linked to a coding sequence if it is positioned to permit translation. Usually, operably linked means contiguous and in reading frame, however, certain genetic elements such as repressor genes are not contiguously linked but still bind to operator sequences that in turn control expression.

Suitable host cells include prokaryotes, lower eukaryotes, and higher eukaryotes. Prokaryotes include both gram negative and gram positive organisms, e.g., *E. coli* and *B. subtilis*. Lower eukaryotes include yeasts, e.g., *S. cerevisiae* and *Pichia*, and species of the genus *Dictyostelium*. Higher eukaryotes include established tissue culture cell lines from animal cells, both of non-mammalian origin, e.g., insect cells, and birds, and of mammalian origin, e.g., human, primates, and rodents.

Prokaryotic host-vector systems include a wide variety of vectors for many different species. As used herein, *E. coli* and its vectors will be used generically to include equivalent vectors used in other prokaryotes. A representative vector for amplifying DNA is pBR322 or many of its derivatives. Vectors that can be used to express these chemokines or their fragments include, but are not limited to, such vectors as those containing the lac promoter (pUC-series); trp promoter (pBR322-trp); Ipp promoter (the pIN-series); lambda-pP or pR promoters (pOTS); or hybrid promoters such as ptac (pDR540). See Brosius, et al. (1988) "Expression Vectors Employing Lambda-, trp-, lac-, and Ipp-derived Promoters," in Rodriguez and Denhardt (eds.) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworth, Boston, Chapter 10, pp. 205-236.

Lower eukaryotes, e.g., yeasts and *Dictyostelium*, may be transformed with chemokine sequence containing vectors. For purposes of this invention, the most common lower eukaryotic host is the baker's yeast, *Saccharomyces cerevisiae*. It will be used to generically represent lower eukaryotes although a number of other strains and species are also available. Yeast vectors typically consist of a replication origin (unless of the integrating type), a selection gene, a promoter, DNA encoding the desired protein or its fragments, and sequences for translation termination, polyadenylation, and transcription termination. Suitable expression vectors for yeast include such constitutive promoters as 3-phosphoglycerate kinase and various other glycolytic enzyme gene promoters or such inducible promoters as the alcohol dehydrogenase 2 promoter or metallothionein promoter. Suitable vectors include derivatives of the following types: self-replicating low copy number (such as the YRp-series), self-replicating high copy number (such as the YEp-series); integrating types (such as the YIp-series), or mini-chromosomes (such as the YCp-series).

Higher eukaryotic tissue culture cells are the preferred host cells for expression of the functionally active chemokine protein. In principle, most any higher eukaryotic tissue culture cell line is workable, e.g., insect baculovirus expression systems, whether from an invertebrate or vertebrate source. However, mammalian cells are preferred, in that the processing, both cotranslationally and posttranslationally.

Transformation or transfection and propagation of such cells has become a routine procedure. Examples of useful cell lines include HeLa cells, Chinese hamster ovary (CHO) cell lines, baby rat kidney (BRK) cell lines, insect cell lines, bird cell lines, and monkey (COS) cell lines. Expression vectors for such cell lines usually include an origin of replication, a promoter, a translation initiation site, RNA splice sites (if genomic DNA is used), a polyadenylation site, and a transcription

termination site. These vectors also usually contain a selection gene or amplification gene. Suitable expression vectors may be plasmids, viruses, or retroviruses carrying promoters derived, e.g., from such 5 sources as from adenovirus, SV40, parvoviruses, vaccinia virus, or cytomegalovirus. Representative examples of suitable expression vectors include pCDNA1; pCD, see Okayama, et al. (1985) Mol. Cell Biol. 5:1136-1142; pMCneo Poly-A, see Thomas, et al. (1987) Cell 51:503-10 512; and a baculovirus vector such as pAC 373 or pAC 610.

It will often be desired to express a chemokine polypeptide in a system which provides a specific or defined glycosylation pattern. In this case, the usual pattern will be that provided naturally by the expression 15 system. However, the pattern will be modifiable by exposing the polypeptide, e.g., an unglycosylated form, to appropriate glycosylating proteins introduced into a heterologous expression system. For example, a chemokine gene may be co-transformed with one or more genes 20 encoding mammalian or other glycosylating enzymes. Using this approach, certain mammalian glycosylation patterns will be achievable or approximated in prokaryote or other cells.

A chemokine, or a fragment thereof, may be 25 engineered to be phosphatidyl inositol (PI) linked to a cell membrane, but can be removed from membranes by treatment with a phosphatidyl inositol cleaving enzyme, e.g., phosphatidyl inositol phospholipase-C. This releases the antigen in a biologically active form, and 30 allows purification by standard procedures of protein chemistry. See, e.g., Low (1989) Biochim. Biophys. Acta 988:427-454; Tse, et al. (1985) Science 230:1003-1008; and Brunner, et al. (1991) J. Cell Biol. 114:1275-1283.

Now that these chemokines have been characterized, 35 fragments or derivatives thereof can be prepared by conventional processes for synthesizing peptides. These include processes such as are described in Stewart and Young (1984) Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL; Bodanszky and Bodanszky

(1984) The Practice of Peptide Synthesis, Springer-Verlag, New York; and Bodanszky (1984) The Principles of Peptide Synthesis, Springer-Verlag, New York. For example, an azide process, an acid chloride process, an acid anhydride process, a mixed anhydride process, an active ester process (for example, p-nitrophenyl ester, N-hydroxysuccinimide ester, or cyanomethyl ester), a carbodiimidazole process, an oxidative-reductive process, or a dicyclohexylcarbodiimide (DCCD)/additive process can be used. Solid phase and solution phase syntheses are both applicable to the foregoing processes.

These chemokines, fragments, or derivatives are suitably prepared in accordance with the above processes as typically employed in peptide synthesis, generally either by a so-called stepwise process which comprises condensing an amino acid to the terminal amino acid, one by one in sequence, or by coupling peptide fragments to the terminal amino acid. Amino groups that are not being used in the coupling reaction are typically protected to prevent coupling at an incorrect location.

If a solid phase synthesis is adopted, the C-terminal amino acid is typically bound to an insoluble carrier or support through its carboxyl group. The insoluble carrier is not particularly limited as long as it has a binding capability to a reactive carboxyl group. Examples of such insoluble carriers include halomethyl resins, such as chloromethyl resin or bromomethyl resin, hydroxymethyl resins, phenol resins, tert-alkyloxycarbonyl-hydrazidated resins, and the like.

An amino group-protected amino acid is bound in sequence through condensation of its activated carboxyl group and the reactive amino group of the previously formed peptide or chain, to synthesize the peptide step by step. After synthesizing the complete sequence, the peptide is split off from the insoluble carrier to produce the peptide. This solid-phase approach is generally described by Merrifield, et al. (1963) in J. Am. Chem. Soc. 85:2149-2156.

The prepared ligand and fragments thereof can be isolated and purified from the reaction mixture by means of peptide separation, e.g., by extraction, precipitation, electrophoresis and various forms of chromatography, and the like. The various chemokines of this invention can be obtained in varying degrees of purity depending upon its desired use. Purification can be accomplished by use of the protein purification techniques disclosed herein or by the use of the antibodies herein described, e.g., in immunoabsorbant affinity chromatography. This immunoabsorbant affinity chromatography is carried out by first linking the antibodies to a solid support and then contacting the linked antibodies with solubilized lysates of appropriate source cells, lysates of other cells expressing the ligand, or lysates or supernatants of cells producing the desired chemokine as a result of DNA techniques, see below.

20 VIII. Uses

The present invention provides reagents which will find use in diagnostic applications as described elsewhere herein, e.g., in the general description for developmental abnormalities, or below in the description of kits for diagnosis.

This invention also provides reagents with significant therapeutic value. These chemokines (naturally occurring or recombinant), fragments thereof and antibodies thereto, along with compounds identified as having binding affinity to them, should be useful in the treatment of conditions associated with abnormal physiology or development, including inflammatory conditions, including asthma. In particular, modulation of trafficking of leukocytes is one likely biological activity, but a wider tissue distribution might suggest broader biological activity, including, e.g., antiviral effects. Abnormal proliferation, regeneration, degeneration, and atrophy may be modulated by appropriate therapeutic treatment using the compositions provided

herein. For example, a disease or disorder associated with abnormal expression or abnormal signaling by a chemokine should be a likely target for an agonist or antagonist of the ligand.

5 Various abnormal physiological or developmental conditions are known in cell types shown to possess the chemokine mRNAs by Northern blot analysis. See Berkow (ed.) The Merck Manual of Diagnosis and Therapy, Merck & Co., Rahway, N.J.; and Thorn, et al. Harrison's
10 Principles of Internal Medicine, McGraw-Hill, N.Y. Developmental or functional abnormalities, e.g., of the immune system, cause significant medical abnormalities and conditions which may be susceptible to prevention or treatment using compositions provided herein.

15 Chemokine antibodies, including recombinant forms, can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active or inert ingredients, e.g., in conventional pharmaceutically acceptable carriers or
20 diluents, e.g., immunogenic adjuvants, along with physiologically innocuous stabilizers and excipients. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This
25 invention also contemplates use of antibodies or binding fragments thereof, including forms which are not complement binding. Moreover, modifications to the antibody molecules or antigen binding fragments thereof, may be adopted which affect the pharmacokinetics or
30 pharmacodynamics of the therapeutic entity.

Drug screening using antibodies or receptor or fragments thereof can be performed to identify compounds having binding affinity to each chemokine or receptor, including isolation of associated components. Subsequent
35 biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks the activity of the ligand. Likewise, a compound having intrinsic stimulating activity can activate the receptor

and is thus an agonist in that it simulates the activity of a chemokine. This invention further contemplates the therapeutic use of antibodies to these chemokines as antagonists. This approach should be particularly useful 5 with other chemokine species variants.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants 10 administered. Thus, treatment dosages should be titrated to optimize safety and efficacy in various populations, including racial subgroups, age, gender, etc. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these 15 reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological 20 Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular 25 administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers typically include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, New Jersey. Dosage ranges would ordinarily be expected to be 30 in amounts lower than 1 mM concentrations, typically less than about 10 μ M concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier. Slow release formulations, or a 35 slow release apparatus will often be utilized for continuous administration.

A chemokine, fragments thereof, or antibodies to it or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or,

depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in any conventional dosage formulation. While it is possible for the active ingredient to be administered alone, it is often preferable to present it as a pharmaceutical formulation. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Carriers may improve storage life, stability, etc.

Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; Avis, et al. (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications Dekker, New York; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Tablets Dekker, New York; and Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, New York. The therapy of this invention may be combined with or used in association with other therapeutic agents.

Both the naturally occurring and the recombinant forms of the chemokines of this invention are particularly useful in kits and assay methods which are capable of screening compounds for binding activity to the proteins. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds in a short period. See, e.g., Fodor, et al. (1991) Science 251:767-773,

which describes means for testing of binding affinity by a plurality of defined polymers synthesized on a solid substrate. The development of suitable assays can be greatly facilitated by the availability of large amounts 5 of purified, soluble chemokine as provided by this invention.

For example, antagonists can normally be found once the ligand has been structurally defined. Testing of potential ligand analogs is now possible upon the 10 development of highly automated assay methods using physiologically responsive cells. In particular, new agonists and antagonists will be discovered by using screening techniques described herein.

Viable cells could also be used to screen for the 15 effects of drugs on respective chemokine mediated functions, e.g., second messenger levels, i.e., Ca^{++} ; inositol phosphate pool changes (see, e.g., Berridge (1993) Nature 361:315-325 or Billah and Anthes (1990) Biochem. J. 269:281-291); cellular morphology 20 modification responses; phosphoinositide lipid turnover; an antiviral response. and others. Some detection methods allow for elimination of a separation step, e.g., a proximity sensitive detection system. Calcium sensitive dyes will be useful for detecting Ca^{++} levels, 25 with a fluorimeter or a fluorescence cell sorting apparatus.

Rational drug design may also be based upon 30 structural studies of the molecular shapes of the chemokines and other effectors or analogs. Effectors may be other proteins which mediate other functions in response to ligand binding, or other proteins which 35 normally interact with the receptor. One means for determining which sites interact with specific other proteins is a physical structure determination, e.g., x-ray crystallography or 2 dimensional NMR techniques. These will provide guidance as to which amino acid residues form molecular contact regions. For a detailed description of protein structural determination, see,

e.g., Blundell and Johnson (1976) Protein Crystallography, Academic Press, New York.

Purified chemokine can be coated directly onto plates for use in the aforementioned drug screening 5 techniques. However, non-neutralizing antibodies to these ligands can be used as capture antibodies to immobilize the respective ligand on the solid phase.

Similar concepts also apply to the chemokine receptor embodiments of the invention.

10

IX. Kits

This invention also contemplates use of chemokine proteins, fragments thereof, peptides, and their fusion products in a variety of diagnostic kits and methods for 15 detecting the presence of ligand, antibodies, or chemokine receptors. Typically the kit will have a compartment containing either a defined chemokine peptide or gene segment or a reagent which recognizes one or the other, e.g., antibodies.

20 A kit for determining the binding affinity of a test compound to a chemokine would typically comprise a test compound; a labeled compound, for example an antibody having known binding affinity for the ligand; a source of chemokine (naturally occurring or recombinant); and a 25 means for separating bound from free labeled compound, such as a solid phase for immobilizing the ligand. Once compounds are screened, those having suitable binding affinity to the ligand can be evaluated in suitable biological assays, as are well known in the art, to 30 determine whether they act as agonists or antagonists to the receptor. The availability of recombinant chemokine polypeptides also provides well-defined standards for calibrating such assays or as positive control samples.

35 A preferred kit for determining the concentration of, for example, a chemokine in a sample would typically comprise a labeled compound, e.g., antibody, having known binding affinity for the ligand, a source of ligand (naturally occurring or recombinant) and a means for separating the bound from free labeled compound, for

example, a solid phase for immobilizing the chemokine. Compartments containing reagents, and instructions for use or disposal, will normally be provided.

Antibodies, including antigen binding fragments, 5 specific for the chemokine or ligand fragments are useful in diagnostic applications to detect the presence of elevated levels of chemokine and/or its fragments. Such diagnostic assays can employ lysates, live cells, fixed cells, immunofluorescence, cell cultures, body fluids, 10 and further can involve the detection of antigens related to the ligand in serum, or the like. Diagnostic assays may be homogeneous (without a separation step between free reagent and antigen-ligand complex) or heterogeneous (with a separation step). Various commercial assays 15 exist, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), enzyme-multiplied immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA), and the like. For example, unlabeled antibodies can be 20 employed by using a second antibody which is labeled and which recognizes the antibody to a chemokine or to a particular fragment thereof. Similar assays have also been extensively discussed in the literature. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, 25 CSH.

Anti-idiotypic antibodies may have similar uses to diagnose presence of antibodies against a chemokine, as such may be diagnostic of various abnormal states. For example, overproduction of a chemokine may result in 30 production of various immunological reactions which may be diagnostic of abnormal physiological states, particularly in various inflammatory or asthma conditions.

Frequently, the reagents for diagnostic assays are 35 supplied in kits, so as to optimize the sensitivity of the assay. For the subject invention, depending upon the nature of the assay, the protocol, and the label, either labeled or unlabeled antibody or labeled chemokine is provided. This is usually in conjunction with other

additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit will also contain instructions for proper use and disposal of the 5 contents after use. Typically the kit has compartments for each useful reagent. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be reconstituted in an aqueous medium providing appropriate concentrations of reagents for performing the 10 assay.

The aforementioned constituents of the drug screening and the diagnostic assays may be used without modification or may be modified in a variety of ways. For example, labeling may be achieved by covalently or 15 non-covalently joining a moiety which directly or indirectly provides a detectable signal. In any of these assays, the ligand, test compound, chemokine, or antibodies thereto can be labeled either directly or indirectly. Possibilities for direct labeling include 20 label groups: radiolabels such as ^{125}I , enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence 25 polarization. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups.

There are also numerous methods of separating bound 30 from the free ligand, or alternatively bound from free test compound. The chemokine can be immobilized on various matrixes followed by washing. Suitable matrixes include plastic such as an ELISA plate, filters, and beads. Methods of immobilizing the chemokine to a matrix 35 include, without limitation, direct adhesion to plastic, use of a capture antibody, chemical coupling, and biotin-avidin. The last step in this approach involves the precipitation of ligand/antibody complex by any of several methods including those utilizing, e.g., an

organic solvent such as polyethylene glycol or a salt such as ammonium sulfate. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described in 5 Rattle, et al. (1984) Clin. Chem. 30:1457-1461, and the double antibody magnetic particle separation as described in U.S. Pat. No. 4,659,678.

Methods for linking proteins or their fragments to the various labels have been extensively reported in the 10 literature and do not require detailed discussion here. Many of the techniques involve the use of activated carboxyl groups either through the use of carbodiimide or active esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an 15 activated halogen such as chloroacetyl, or an activated olefin such as maleimide, for linkage, or the like. Fusion proteins will also find use in these applications.

Another diagnostic aspect of this invention involves use of oligonucleotide or polynucleotide sequences taken 20 from the sequence of a chemokine. These sequences can be used as probes for detecting levels of the ligand message in samples from patients suspected of having an abnormal condition, e.g., an inflammatory or developmental problem. The preparation of both RNA and DNA nucleotide 25 sequences, the labeling of the sequences, and the preferred size of the sequences has received ample description and discussion in the literature. Normally an oligonucleotide probe should have at least about 14 nucleotides, usually at least about 18 nucleotides, and 30 the polynucleotide probes may be up to several kilobases. Various labels may be employed, most commonly radionuclides, particularly ^{32}P . However, other 35 techniques may also be employed, such as using biotin modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed which can recognize specific

duplexes, including DNA duplexes, RNA duplexes, DNA-RNA hybrid duplexes, or DNA-protein duplexes. The antibodies in turn may be labeled and the assay carried out where the duplex is bound to a surface, so that upon the 5 formation of duplex on the surface, the presence of antibody bound to the duplex can be detected. The use of probes to the novel anti-sense RNA may be carried out in any conventional techniques such as nucleic acid hybridization, plus and minus screening, recombinational 10 probing, hybrid released translation (HRT), and hybrid arrested translation (HART). This also includes amplification techniques such as polymerase chain reaction (PCR).

Diagnostic kits which also test for the qualitative 15 or quantitative presence of other markers are also contemplated. Diagnosis or prognosis may depend on the combination of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet, et al. (1989) Progress in Growth Factor 20 Res. 1:89-97.

X. Receptor

Having isolated a ligand binding partner of a specific interaction, methods exist for isolating the 25 counter-partner. See, Gearing, et al EMBO J. 8:3667-4676 or McMahan, et al. (1991) EMBO J. 10:2821-2832. For example, means to label a chemokine without interfering with the binding to its receptor can be determined. For example, an affinity label can be fused to either the 30 amino- or carboxy-terminus of the ligand. An expression library can be screened for specific binding of chemokine, e.g., by cell sorting, or other screening to detect subpopulations which express such a binding component. See, e.g., Ho, et al. (1993) Proc. Nat'l Acad. Sci. 90:11267-11271. Alternatively, a panning 35 method may be used. See, e.g., Seed and Aruffo (1987) Proc. Nat'l. Acad. Sci. 84:3365-3369.

Protein cross-linking techniques with label can be applied to isolate binding partners of a chemokine. This

would allow identification of protein which specifically interacts with a chemokine, e.g., in a ligand-receptor like manner.

In various embodiments, new receptors designated DC 5 CR and M/DC CR were isolated. The sequences of the human constructs are set forth in SEQ ID NOS: 10 and 12. Similar means for making variants and fragments, at the 10 nucleotide level or at the protein level, and making antibodies will be available as described above, directed primarily to the chemokine embodiments. Many similar or 15 related uses to the ligands will be applied to the receptors, as specific binding reagents. In particular, methods will be applied to screening for specific ligands for each receptor. Many uses, including kits, will also be available through analogous techniques.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the invention to specific embodiments.

20

EXAMPLES

I. General Methods

Some of the standard methods are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning. A Laboratory Manual, Cold Spring Harbor 25 Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols 1-3, CSH Press, NY; Ausubel, et al., Biology, Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. 30 (1987 and Supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York; Innis, et al. (eds.) (1990) PCR Protocols: A Guide to Methods and Applications Academic Press, N.Y. Methods for protein purification include such methods as ammonium sulfate 35 precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification" in Methods in Enzymology, vol. 182, and other volumes in

this series; and manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA. Combination with recombinant techniques allows fusion to appropriate segments, e.g., to a FLAG sequence or an equivalent which can be fused via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 12:69-70; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods 12:87-98, Plenum Press, N.Y.; and Crowe, et al. (1992) QIAexpress: The High Level Expression & Protein Purification System QUIAGEN, Inc., Chatsworth, CA.

FACS analyses are described in Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY.

20 II. Isolation and characterization of chemokine cDNAs
A. TECK

The TECK was isolated from a cDNA library made from thymus cells from a RAG-1 "knockout" mouse. See, Mombaerts, et al. (1992) Cell 68:869-877. Individual cDNA clones were sequenced using standard methods, e.g., the Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA), and the TECK sequence was identified and further characterized. Computer analyses with other C-C chemokine family members revealed significant homology at the amino acid levels with other chemokines. The nucleotide sequence for mouse is provided in SEQ ID NO: 1, encoding a polypeptide of about 144 amino acids. The signal sequence should run from 1 (met) to about 23 (ala), and removal of the signal sequence should provide one natural mature sequence beginning at 24 (gln). Additional processing may occur in a physiological system.

The sequence is notable in having a longer carboxy-terminal tail than most other CC chemokines. TECK

exhibits one glycosylation site, and several AAMP, PKC, and CK2 phosphorylation sites.

B. MIP-3 α

The MIP-3 α was isolated from a cDNA library made from human monocytes activated with LPS and IFN- γ . Individual cDNA clones were sequenced using standard methods, and the MIP-3 α sequence was identified and further characterized. The nucleotide sequence is set forth in SEQ ID NO: 5, encoding a polypeptide of at least about 89 amino acids. The signal sequence should run from about 1 (met) to 21 (cys), and removal of the signal sequence should provide one natural sequence beginning with gly. Additional processing may occur in a physiological system.

15 C. MIP-3 β

The MIP-3 β was isolated from a cDNA library made from human fetal lung cells. Individual cDNA clones are sequenced using standard methods, and the MIP-3 α sequence was identified and further characterized. The nucleotide sequence is set forth in SEQ ID NO: 7, encoding a polypeptide of about 98 amino acids. The signal sequence should run from about 1 (met) to about 21 (ser), and removal of the signal sequence should provide one mature natural sequence beginning from gly. Additional processing may occur in a physiological system.

D. Dendritic Cell Receptor for chemokine; DC CR

The DC CR was isolated from RNA made from dendritic cells isolated from CD34 $^{+}$ cord blood cells, isolated by standard procedure. It was also isolated from 30 eosinophils using degenerate PCR primers of the TM2 and TM7 segments, which are often conserved among chemokine receptors. These eosinophils were isolated by taking PBLs, depletion of red blood cells by lysis, and negative selection of CD16 to remove neutrophils.

35 Sequencing of the PCR fragments indicated a potential novel receptor, and the fragment was used to isolate a full length clone by hybridization. Clone isolates were sequenced using standard methods, and the DC CR sequence was identified and further characterized.

The nucleotide sequence is provided in SEQ ID NO: 9, encoding a polypeptide of about 365 amino acids. The transmembrane segments, determined by homology to the IL-8 B receptor, are about: TM1 from 39 (leu) to 64 (phe); 5 TM2 from 76 (leu) to 96 (ser); TM3 from 111 (leu) to 132 (met); TM4 from 151 (thr) to 176 (phe); TM5 from 207 (gly) to 229 (val); TM6 from 246 (val) to 270 (ala); and TM7 from 291 (val) to 319 (leu). The amino terminal segment is probably an extracellular segment, and the 10 others would be between TM2 and TM3; and TM4 and TM5; and TM6 and TM7. The intracellular segments should then run between TM1 and TM2; TM3 and TM4, TM5 and TM6, and the carboxy terminus from the end of TM7. Additional processing may occur in a physiological system.

15 The implication of chemokine receptors in retroviral infection suggests that the receptor may be critical for infection. Antibodies which block infection may be routinely screened, and developed for therapeutic uses.

20 E. Monocyte/Dendritic Cell Receptor for chemokine;
M/DC CR

The M/DC CR was isolated from a cDNA library made from human monocyte cells cultured for 2.5 to 5 h in medium containing IFN- γ (10 ng/ml), LPS (1 μ g/ml), anti-IL-4 monoclonal antibody (5 μ g/ml), and anti-IL-10 25 monoclonal antibody (5 μ g/ml). Individual cDNA clones were sequenced using standard methods, and the M/DC CR sequence was identified and further characterized. The nucleotide sequence is set forth in SEQ ID NO: 11, encoding a polypeptide of about 356 amino acids. The 30 transmembrane segments, should be about as follows: TM1 from 52 (leu) to 76 (val); TM2 from 86 (asn) to 107 (ala); TM3 from 117 (ile) to 138 (val); TM4 from 157 (val) to 182 (tyr); TM5 from 211 (phe) to 233 (val); TM6 from 251 (leu) to 275 (phe); and TM7 from 296 (ile) to 35 TM7 from 296 (ile) to 315 (leu). As for the DC CR, the amino terminal segment is probably an extracellular segment, and the others would be between TM2 and TM3; and TM4 and TM5; and TM6 and TM7. The intracellular segments should then run

between TM1 and TM2; TM3 and TM4, TM5 and TM6, and the carboxy terminus from the end of TM7.

III. Preparation of antibodies

5 Many standard methods are available for preparation of antibodies. For example, synthetic peptides may be prepared to be used as antigen, administered to an appropriate animal, and either polyclonal or monoclonal antibodies prepared. Short peptides, e.g., less than
10 about 10 amino acids may be repeated, while longer peptides may be used alone or conjugated to a carrier. For example, with the M/DC CR, animals were immunized with peptides corresponding to amino acid sequences from 18-44 (starting with LAP and ending with KYD; a fragment 15 towards the amino terminus) and from 183-204 (starting with KPQ and ending with PAD; corresponding to an extracellular loop), see SEQ ID NO: 13. Highest specificity will result when the polypeptides are selected from portions which are most unique, e.g., not
20 form conserved sequence regions. The animals may be used to collect antiserum, or may be used to generate monoclonal antibodies.

Antiserum was determined useful for ELISA, and will be evaluated for utility as immunoprecipitation or
25 Western blot analysis. Monoclonal antibodies will also be evaluated for those same uses.

The antibodies provided will be useful as immunoaffinity reagents, as detection reagents, for immunohistochemistry, and as therapeutic reagents.

30

IV. Assays for chemotactic activity of chemokines.

Chemokine proteins are produced, e.g., in COS cells transfected with a plasmid carrying the chemokine cDNA by electroporation. See, Hara, et al. (1992) EMBO J.
35 10:1875-1884. Physical analytical methods may be applied, e.g., CD analysis, to compare tertiary structure to other chemokines to evaluate whether the protein has likely folded into an active conformation. After transfection, a culture supernatant is collected and

subjected to bioassays. A mock control, e.g., a plasmid carrying the luciferase cDNA, is used. See, de Wet, et al. (1987) Mol. Cell. Biol. 7:725-757. A positive control, e.g., recombinant murine MIP-1 α from R&D Systems (Minneapolis, MN), is typically used. Likewise, antibodies may be used to block the biological activities, e.g., as a control.

5 Lymphocyte migration assays are performed as previously described, e.g., in Bacon, et al. (1988) Br. 10 J. Pharmacol. 95:966-974. Murine Th2 T cell clones, CDC-25 (see Tony, et al. (1985) J. Exp. Med. 161:223-241) and HDK-1 (see Cherwinski, et al. (1987) J. Exp. Med. 166:1229-1244), made available from R. Coffman and A. O'Garra (DNAZ, Palo Alto, CA), respectively, are used as 15 controls.

Ca²⁺ flux upon chemokine stimulation is measured according to the published procedure described in Bacon, et al. (1995) J. Immunol. 154:3654-3666.

20 Maximal numbers of migrating cells in response to MIP-1 α typically occur at a concentration of 10⁻⁸ M, in agreement with original reports for CD4+ populations of human T cells. See Schall (1993) J. Exp. Med. 177:1821-1826. A dose-response curve is determined, 25 preferably giving a characteristic bell shaped dose-response curve.

After stimulation with C-C chemokines, lymphocytes generally show a measurable intracellular Ca²⁺ flux. MIP-1 α is capable of inducing immediate transients of calcium mobilization. Typically, the levels of chemokine 30 used in these assays will be comparable to those used for the chemotaxis assays (1/1000 dilution of conditioned supernatants).

35 Retroviral infection assays have also been described, and recent description of certain chemokine receptors in retroviral infection processes may indicate that similar roles may apply to the DC CR and/or M/DC CR. See, e.g., Balter (1996) Science 272:1740 (describing evidence for chemokine receptors as coreceptors for HIV); and Deng, et al. (1996) Nature 381:661-666.

V. Expression analysis of chemokine/receptor genes

RNA blot and hybridization are performed according to the standard method in Maniatis, et al. (1982) Molecular Cloning: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. An appropriate fragment of a cDNA fragment is selected for use as a probe. To verify the amount of RNA loaded in each lane, the substrate membrane is reprobed with a control cDNA, e.g., glyceraldehyde 3-phosphate dehydrogenase (G3PDH) cDNA (Clontech, Palo Alto CA). Analysis of mRNA from the appropriate cell source using the probe will determine the natural size of message. It will also indicate whether different sized messages exist. The messages will be subject to analysis after isolation, e.g., by PCR or hybridization techniques. Northern blot analysis may be performed on many different mRNA sources. However, in certain cases, cDNA libraries may be used to evaluate sources which are difficult to prepare. A "reverse Northern" uses cDNA inserts removed from vector, but multiplicity of bands may reflect either different sized messages, or may be artifact due to incomplete reverse transcription in the preparation of the cDNA library. In such instances, verification may be appropriate by standard Northern analysis. Similarly, Southern blots may be used to evaluate species distribution of a gene. The stringency of washes of the blot will also provide information as to the extent of homology of various species counterparts.

Tissue distribution, and cell distribution, may be evaluated by immunohistochemistry using antibodies. Alternatively, *in situ* nucleic acid hybridization may also be used in such analysis.

A. TECK

The TECK was isolated from a RAG-1 "knockout" mouse. This animal is characterized by a great predominance of pro-T or pre-T cells, lacking more mature T cells after the block point of T cell receptor rearrangement. This suggests a role in very early T cell development, likely expressed by pro-T or pre-T cells, thymic stromal cells, and possibly macrophages, epithelial, and dendritic cells. This comports with the observation that tissue distribution

Table 2: mTECK mRNA expression in tissues and cells

cDNA libraries			northern blot		
cell type or tissue	neg	pos	cell type or tissue	neg	pos
Th2 CD4+ T cells	X		heart		X
Th1 CD4+ T cells	X		brain		X
Lung	X		spleen		X
L cells	X		lung		X
RAG-1 KO lung	X		liver		X
RAG-1 KO heart	X		skeletal muscle		X
RAG-1 KO brain		X (+)	kidney		X
RAG-1 KO spleen	X		testis		X
RAG-1 KO kidney	X		thymus		
RAG-1 KO testis		X (+)	small intestine		X (+++)
RAG-1 KO thymus		X (+++)	CD4+8- thymocytes	R/A	X (++)
RAG-1 KO liver		X (+)	CD4+8- thymocytes	R/A	X
CD4-8- thymocytes	X		CD4-8- thymocytes	R/A	X
A20-J B-cell lymphoma	X		B220+ splenocytes	R/A	X
BW CD4-8-3- hybridoma	X		Thy-1+ splenocytes	R/A	X
pro-T cells		X (+)	IG18LA macrophages	R/A	X
pre-T cells	X		primary thymic stroma	R/A	X
30-R bone marrow stroma	X		3D.1 thymic epithelial	R/A	X
D10 T-cell hybridoma	X		MTSC-C thymic epithelial		X
CTLL T-cell clone	X		30.R bone marrow stroma		X
peritoneal macrophages	X				
splenic dendritic cells	X				

Analysis of mTECK mRNA was carried out as described. + to +++ indicates the relative intensity of the signal. R/A: resting or activated.

Species analysis indicated positive signals by hybridization in human, rat, and hamster DNA. Tissue distribution analysis suggests that the gene is expressed in human small intestine, which also is a tissue which 5 supports T cell differentiation.

The combination of the structure and distribution of this chemokine suggests a role in T cell development, which normally occurs in the thymus.

B. MIP-3 α

10 The MIP-3 α was identified from a cDNA library made from human monocytes activated with LPS and IFN- γ , in the presence of anti-IL-10. It has also been detected in pancreatic islet cells, fetal lung, and hepatic HEPG2 cells.

15 The gene is expressed in HL-60 (promyelocytic leukemia); S3 (HeLa cell); K562 (chronic myelogenous leukemia); MOLT-4 (lymphoblastic leukemia); Raji

(Burkitt's lymphoma); SW480 (colorectal adenocarcinoma); A549 (lung carcinoma); and G361 (melanoma) cell lines, as determined by probing on a tissue blot from CLONTECH. Tissue expression gave a positive signal in lymph node, 5 appendix, peripheral blood lymphocytes, fetal liver, and fetal lung; but no detectable signal in spleen, bone marrow, brain, and kidney.

The main transcript appears to be about 1.2 kb, with two additional transcript sizes in fetal lung RNA. Among 10 the various tissues, transcript sizes of 1.8, 2.7, and 4.2 kb were detected.

Positive signals were also detected in the following cDNA libraries: dendritic cells activated with LPS, but not when activated with GM-CSF and IL-4; monocytes 15 treated with LPS, IFN- γ , and anti-IL-10, but not when treated with LPS, IFN- γ , and IL-10; and activated PBMC.

These expression data implicate this chemokine in inflammatory responses upon cell activation. The lymph nodes, appendix, and PBL are sites where inflammatory 20 processes take place. The MIP-3 α may exert its effects on monocytes and cells involved in inflammatory events. Other structural features implicate this chemokine in eosinophil and lung physiology, e.g., asthma indications. Thus, an antagonist of the chemokine, e.g., an antibody, 25 may be important for treatment of asthmatic conditions. Also, IL-10 appears to inhibit MIP-3 α expression.

The human MIP-3 α is a ligand for the DC CR. Thus, a positive control exists for the Ca $^{++}$ flux assay for 30 that receptor. This allows for the further screening of agonist ligands for the DC CR. Moreover, the DC CR was isolated from eosinophil cDNA, and observations have been made that eosinophils migrate to MIP-3 α in vitro. This suggests that the MIP-3 α interaction with the DC CR is important in recruitment of eosinophils, as occurs with 35 the eotaxin ligand and the CCR3. As such, antagonists of the MIP-3 α interaction with the DC CR will likely be useful in inhibiting eosinophilia, particularly in the lung, or lung inflammation. These may accompany asthmatic or other pulmonary conditions.

Antagonists to MIP-3 α may be made either with antibodies, or other binding compositions which inhibit receptor interaction. The antibodies may be to the ligand, MIP-3 α itself, or to the binding portions of the

5 receptor, DC CR. Muteins of the chemokine may block receptor interaction, and with a positive control, chemokine muteins may be screened for variations which compete with the wild type chemokine at various concentrations. See, e.g., Kenakin (1987)

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Raven Press, NY; Arunlakshana and Schild (1959) Br. J. Pharmacol. 14:48-58; Black (1989) Science 245:486-493; Zurawski, et al. (1986) J. Immunol. 137:3354-3360; Zurawski and Zurawski (1988) EMBO J. 7:1061-1069; 15 Zurawski and Zurawski (1992) EMBO J. 11:3905-3910; Imler and Zurawski (1992) J. Biol. Chem. 267:13185-13190.

C. MIP-3 β

20 The MIP-3 β was identified in a cDNA library made from human monocytes activated with LPS and IFN- γ , in the presence of anti-IL-10. Its distribution in other cells and tissues has not been fully determined.

D. Dendritic Cell Receptor for chemokine; DC CR

25 The DC CR was isolated from a cDNA library made from a dendritic cell cDNA library. It appears to be expressed in certain T cells, spleen cell subsets, NK cells, and other cell populations enriched in dendritic cells, including CD1a $^+$, CD14 $^+$, and CD1Aa $^+$ cells. It did not give a detectable signal in TF1, Jurkat, MRC5, JY, or U937 cells.

30 Being found on dendritic cells, its ligand, including the MIP-3 α , may be important in attracting appropriate cells for the initiation of an immune response. MIP-3 α is therefore likely to attract dendritic cells, leading to initiation of the immune response. Pulmonary physiology is suggested, both from the distribution of the receptor and ligand. The receptor may be also present in other cells important in such responses.

E. Monocyte/Dendritic Cell Receptor for chemokine;
M/DC CR

The M/DC CR was isolated from a cDNA library made from primary monocyte cells activated with LPS and IFN- γ

5 but subtracted with known high abundance genes from those cells. The abundance of this gene is probably less than about 1% of message from those cells.

Tissue expression gave a positive signal in spleen, PBL, lung, placenta, and small intestine; but no 10 detectable signal in brain, liver, kidney, and muscle. This distribution suggests a hematopoietic role.

There appears to be one main transcript, but the existence of additional or alternatively spliced messages has not been eliminated.

15 Positive signals were also detected in the following cDNA libraries: monocytes and dendritic cells; but signals were not detectable in CD8 $^{+}$ T cells, or in either resting or activated splenocytes, gamma-delta T cells, NK cells, or B cells. Immunohistochemistry will be 20 performed to confirm absence in the T cell and B cell compartments and to check in tonsil, particularly in view of location in spleen and placenta. The relatively restricted distribution on monocytes and dendritic cells leads both to its designation, and suggests a functional 25 role in those cell types, which are important in the initiation of immune responses through their ability to process and present antigen to T cells.

VI. Specific Characterization of TECK

30 A novel CC chemokine was identified in the thymus of mouse and human and was designated TECK as Thymus Expressed ChemoKine. TECK has weak homology with other CC chemokines and maps to mouse chromosome 8. Besides the thymus, mRNA encoding TECK was detected at 35 substantial levels in the small intestine and at low levels in the liver. The source of TECK in the thymus was determined to be thymic dendritic cells, while in contrast bone marrow-derived dendritic cells do not express TECK. The murine TECK recombinant protein showed

chemotactic activity for activated macrophages, dendritic cells and thymocytes. We conclude that TECK represents a novel thymic dendritic cell-specific CC chemokine which is possibly involved in T-cell development.

5 Chemokines belong to a family of small peptides (6-15 kDa) whose best described biological function is to control the migration of certain leukocyte populations to localized sites of inflammation. Baggiolini, et al. (1994) Adv. in Immun. 55:97-179; Schall and Bacon (1994) Curr Opin Immun 6:865-873; Hedrick and Zlotnik (1996) Curr. Opin. Immunol. 8:343-347. In the last few years many new members of the chemokine super family have been characterized. Initially, new chemokines were identified through their chemotactic effects on 10 leukocytes (Baggiolini et al. (1994); Schall and Bacon (1994)) and were isolated mainly from blood leukocytes or cell lines. More recently, approaches based on the selective cloning of secreted molecules by signal sequence trap (Tashiro, et al. (1993) Science 261:600-603; Imai, et al. (1996) J. Biol. Chem. 271:21514-21521) or on the exploitation of public and private databases of expressed sequence tags (EST) through bioinformatics (Hieshima, et al. (1997) J. Biol. Chem. 272:5846-5853; Patel, et al. (1997) J. Exp. Med. 185:1163-1172; and 15 Rossi, et al. (1997) J. Immunol. 158:1033-1036), have allowed the rapid identification of novel chemokines based on sequence and structural homologies. These approaches take advantage of the fact that most of the chemokines are secreted factors whose protein sequence 20 contain four conserved cysteines (Schall (1994) "The Chemokines" pp. 419-460 in Thomson (eds.) The Cytokine Handbook, Academic Press, New York. The CXC or a chemokine family has the two first amino-terminal cysteines separated by a non-conserved amino acid. In 25 the CC or β chemokine family, these two cysteines are consecutive. A third type of chemokine, the C or γ family, is represented by lymphotoxin, which conserves two cysteines (1 and 3) instead of the original four (Kelner, et al. (1994) Science 266:1395-1399). Finally,

a recently identified chemokine with three amino acids separating the first two cysteines defines a fourth CX₃C family (Bazan, et al. (1997) Nature 385:640-644).

Interestingly, some of the new chemokines discovered 5 show a relatively restricted pattern of expression (Imai et al. (1996); Hieshima et al. (1997)). These new approaches may lead to the discovery of tissue- or cell-specific chemokines. In addition, new biological 10 evidence exists for important new roles of chemokines in hemopoiesis (Cook (1996) J. Leukoc. Biol. 59:61-66; and Nagasawa, et al. (1996) Nature 382:635-638) and the control of viral infections including HIV (Cocchi, et al. 15 (1995) Science 270:1811-1815; and Cook, et al. (1995) Science 269:1583-1585). Thus, the molecular cloning of novel chemokines through DNA-based strategies may uncover 20 novel proteins belonging to the chemokine super family but whose physiological role goes beyond the control of inflammation.

In an attempt to identify novel genes involved in T-cell development, we analyzed a cDNA library from the 25 thymus of Recombinase Activation Gene-1 (RAG-1) deficient mice. We identified a novel CC chemokine designated TECK for Thymus Expressed ChemoKine, based on sequence homology with other known chemokines. We subsequently isolated the human homologue of TECK. The pattern of 30 expression of TECK mRNA is highly restricted to the thymus and small intestine in both human and mouse. Moreover, in the mouse thymus, TECK protein is produced by dendritic cells while splenic dendritic cells do not 35 express TECK mRNA. Recombinant TECK showed chemotactic activity on thymocytes, macrophages, THP-1 cells and dendritic cells, while it was inactive on peripheral lymphocytes and neutrophils. The restricted pattern of expression of TECK together with its biological properties suggests a role for this novel dendritic cell-specific chemokine in T-cell development.

A. Cloning and structural analysis of mouse TECK

A directional cDNA library was made from RAG-1 deficient mouse thymus and analyzed by random sequencing.

One of the clones contained an open reading frame with significant homology to previously described CC chemokines. The full-length cDNA contains 1037 bp including an open reading frame of 426 bp encoding a 5 protein of 142 amino acids and will be identified in this report as mTECK (see SEQ ID NO: 1). In the 3' untranslated region, there is one unique polyadenylation signal consistent with the single mRNA species observed in northern blots. The mTECK cDNA does not contain any 10 ATTAA transcript destabilization motif (Shaw and Kamrn (1986) *Cell* 46:659-667). The comparison of the amino acid sequence of mTECK with previously described murine CC chemokines demonstrates the conservation of the four 15 cysteines present in all these chemokines. However, mTECK shows few additional identities with these proteins.

B. Cloning and molecular characterization of human TECK

To investigate the possible existence of a gene homologous to mTECK in other mammalian species, a

20 Southern blot with genomic DNA from various species was hybridized with the mTECK cDNA probe. Under high stringency conditions, hybridizing bands were detected in mouse, rat, hamster and human genomic DNAs.

Interestingly, a single band was detected in human,

25 suggesting that a single gene encodes for TECK in this species. The multiple bands present in mouse, rat and hamster could be the result of an internal EcoRI site within the TECK gene. Alternatively, the TECK gene may have been duplicated in these species.

30 In order to clone the human homologue of mTECK, a blot of cDNAs from a panel of human cDNA libraries was hybridized with the mTECK cDNA probe. A signal was observed in a fetal small intestine cDNA library.

Screening of this library with the mTECK probe allowed

35 the isolation of several identical clones of 1012 bp with an open reading frame of 453 bp encoding a protein of 151 amino acids. This protein had a much higher degree of homology at the nucleic acid level (71% nucleic acid identity for the open reading frame and 49.3% amino acid

identity) to mTECK than to other known CC chemokines and was thus designated as hTECK.

C. Chromosomal location of mTECK

It has been shown that the genes encoding for most chemokines are clustered in the genome. The genes encoding CC chemokines cluster on mouse chromosome 11 and human chromosome 17q11-12. Schall (1994); and Hedrick and Zlotnik (1996). The chromosomal location of mTECK (designated gene symbol Teck) was determined by inter specific backcross analysis between ([C57Bl/6J X M. spreatus]F1 X C57Bl/6J) mice. Jenkins, et al. (1982) J. Virol. 43:26-36. The mapping results indicated that the Teck locus is located on the proximal part of mouse chromosome 8. Although the chromosomal location of the human Teck locus could not be determined, this region of mouse chromosome 8 is syntenic to the human 19p13.3 and 13q34 regions. However, the Teck locus is also very close to a region syntenic to human chromosome 4. The closest known gene, Insr, encodes the insulin receptor and the genetic distance between Teck and Insr was estimated at 0.9 ± 0.9 cM. We have compared our inter specific map of chromosome 8 with a composite mouse linkage map that reports the map location of many uncloned mouse mutations (Mouse Genome Database, The Jackson Laboratory, Bar Harbor, ME). Teck resides in a region of the composite map that lacks mouse mutations with a phenotype that might be expected for an alteration in this locus.

D. Analysis of mTECK and hTECK mRNA distribution in cells and tissues

An analysis of the distribution of mTECK mRNA in tissues and cells by northern blotting or by Southern blotting of mouse cDNA libraries revealed that mTECK was expressed at significant levels only in the thymus and to a lesser extent in small intestine (Table 2). Weak expression of mTECK mRNA was observed in brain, testis, and liver RAG-1-/- cDNA libraries. Interestingly, mTECK mRNA was detected in a cDNA library of activated pro-T cells. Pro-T cells represent an early stage of intra-

thymic T-cell progenitors, not fully committed to the T-cell lineage since they can give rise to NK and dendritic cells. Moore and Zlotnik (1995) *Blood* 86:1850-1860; Wu, et al. (1996). *J. Exp. Med.* 184:903-911). In contrast, 5 mTECK mRNA was undetectable in resting or activated thymocytes, peripheral T or B cells, macrophages, PBLs, splenic dendritic cells and in all other tissues tested, with the exception of spleens recovered from mice injected with LPS (Table 2). Interestingly, mTECK mRNA 10 was detected by PCR in fetal thymuses of day 14 of gestation, indicating that mTECK is expressed in the thymus at the earliest stages of T-cell development.

The distribution of hTECK mRNA was similarly analyzed. As with mouse, hTECK mRNA expression was 15 highly restricted to the thymus and small intestine. Weak expression was also detected in inflamed tonsil and fetal spleen, but at much lower levels than that observed in the thymus since this particular blot was exposed for a long time. Importantly, hTECK mRNA was absent from a 20 series of cDNA libraries from dendritic cells derived in vitro from bone marrow CD34+ progenitors cells or peripheral blood monocytes. In addition, hTECK mRNA was also absent from libraries of monocyte-derived dendritic cells stimulated with LPS or a combination of TNF- α , IL-25 1 α and monocyte supernatant for 4 and 16 hours.

Collectively, these data indicate that TECK mRNA is specifically expressed at high levels in thymus and small intestine in vivo.

E. Identification of mTECK-producing cells in vivo

30 The abundance of mTECK mRNA expression in RAG-1 deficient thymus and its absence in thymic T cells suggested that mTECK was expressed by a thymic stromal component in normal mice. We performed *in situ* mRNA hybridization with sense or antisense mTECK probes 35 generated by PCR. Thymic sections hybridized with the sense probe (negative control) demonstrated no specific staining while sections hybridized with the anti-sense probe at the same concentration showed specific staining in the thymic medulla. At higher magnification, positive

cells appeared to have a non-lymphoid morphology with processes surrounding lymphoid cells. This experiment indicated that, *in vivo*, mTECK mRNA is expressed by a non-lymphoid component of the medullary stroma, possibly 5 dendritic cells.

The thymic stroma is mainly composed of epithelial cells, macrophages, dendritic cells and fibroblasts, together with a network of vascular and nervous tissue. Boyd, et al. (1993) Immunol. Today 14:445-459. Since we 10 previously failed to detect mTECK mRNA expression in thymic epithelial or macrophage cell lines with our without activation with IFN- γ (Table 2), we sorted thymic dendritic cells based on their high expression of MHC class II and CD11c (N-418 antibody). Analysis of mTECK 15 expression by RT-PCR revealed that freshly isolated MHC class II+ CD11c+ thymic dendritic cells expressed mTECK mRNA while the MHC class II+ CD11c- subset was negative. In contrast, mTECK mRNA was undetectable in a cDNA library made from freshly isolated splenic dendritic 20 cells (Table 2).

We then performed immunostaining of thymic sections and purified thymic dendritic cells with a polyclonal antibody raised against a decapeptide corresponding to the C-terminus of mTECK. This polyclonal antibody 25 reacted with recombinant mTECK in ELISA and western blot while normal rabbit serum was negative. In thymic sections, the polyclonal anti-peptide antibody reacted with a stromal component of the thymic medulla consistent with the *in situ* hybridization data while staining with 30 normal rabbit serum was negative. Interestingly, the antibody also reacted weakly with some endothelial cells, raising the possibility that mTECK can be produced by the thymic endothelium. Finally, the anti-mTECK polyclonal antibody stained sorted thymic dendritic cells, while the 35 control serum was negative. High magnification clearly showed intra-cellular staining of cells with characteristic dendritic morphology. Taken together, these results indicate that thymic dendritic cells and

possibly thymic endothelial cells are producing TECK in vivo.

F. Chemotactic activities of mTECK protein

To evaluate the biologic properties of mTECK, a recombinant protein with an N-terminal FLAG peptide was obtained in a bacterial expression system. In some experiments, a recombinant mTECK protein with a C-terminal FLAG was used and similar results were obtained. Interestingly, mTECK induced the migration of mouse thymocytes (Figure 1A). The optimal response was obtained with a dose of 10 ng/ml TECK. Cell migration was determined to be chemotaxis and not chemokinesis through the checkerboard analysis. Furthermore, it is established that chemokines bind to specific receptors that are coupled through heterotrimeric G proteins to intra-cellular signal-transducing pathways. Murphy (1994) *Annu. Rev. Immunol.* 12:593-633. To determine whether the chemotaxis of thymocytes involved a G protein-coupled receptor, cells were incubated prior to the assay with 10 ng/ml pertussis toxin which ADP-ribosylates Gαi-proteins. Katz, et al. (1992) *Nature* 360:686-689. This pre-treatment completely abrogated the chemotactic response of thymocytes to mTECK (Figure 1A).

The recombinant mTECK protein also induced the migration of human monocytic THP-1 cells activated for 16 hours with IFN-γ (Figure 1B), while it was not significantly active on resting THP-1 cells. This experiment showed that mTECK is active on human cells. In addition, mTECK induced activated mouse peritoneal macrophages to migrate as well as highly purified mouse splenic dendritic cells (Figure 1B). In all these experiments, the optimal dose of mTECK was 10 ng/ml. In contrast, no chemotaxis was observed with bone marrow cells, purified neutrophils, splenic B cells, splenic T cells or IL-2 activated RAG-1 deficient mouse splenocytes lacking mature T and B lymphocytes (Mombaerts, et al. (1992) *Cell* 68:869-877) and therefore enriched in NK cells. These data are consistent with the absence of in vivo accumulation of neutrophils, monocytes or

lymphocytes 2 and 5 h following an intra-peritoneal injection of 10 µg mTECK. Collectively, these data indicate that TECK is a chemotactic factor for thymocytes, macrophages and dendritic cells.

5 G. TECK, a distant member of the CC chemokine family
In this report, we describe the molecular isolation and characterization of TECK, a novel mouse and human CC chemokine. Analysis of its predicted amino acid sequence showed that TECK is distantly related to previously
10 described CC chemokines. Conservation of particular amino acids among most CC chemokines may be related to their functional importance. Beall, et al. (1992) *J. Biol. Chem.* 267:3455-3459; and Lusti-Narasimhan, et al. (1995) *J. Biol. Chem.* 270:2716-2721. In particular, a
15 tyrosine residue between the second and third cysteines has been shown to be critical for monocyte chemotaxis (in position 50) (Beall et al. (1992)). While TECK does not have a tyrosine at this particular position, it has one in position 52 that may have the same function, since
20 TECK is chemotactic for activated monocytes. In addition to these differences in the primary structure, the gene encoding TECK maps on chromosome 8 in the mouse, unlike most other CC chemokines which are clustered on chromosome 11. This is not the first report of an
25 unusual chromosomal location for a CC chemokine. We have cloned the human CC chemokine MIP-3 β and showed that its gene was on chromosome 9 rather than 17 (Rossi, et al. (1997)), and the gene encoding the novel human CC chemokine MIP-3 α /LARC (Rossi, et al. (1997)) has been
30 mapped on chromosome 2 (Hieshima, et al. (1997)). It is likely that the CC chemokines on chromosome 11 in the mouse and 17 in human have been generated through gene duplication of a primordial chemokine. Our results suggest that TECK may have been generated at an earlier
35 stage during evolution. In this regard, the TECK gene may have evolved to ensure functions similar to other CC chemokines with a distant primary structure but through similar receptor(s) as dictated by its secondary and tertiary structures. Alternatively, the receptor(s) and

physiological role of TECK may be unique among chemokines.

H. TECK expression and function is associated with T-cell development

5 We observed that TECK was strongly expressed in the thymus which is the primary lymphoid organ where T-cell development takes place. Recently, another CC chemokine highly expressed in the thymus, TARC, has been identified. Imai, et al. (1996). However, TARC is also
10 expressed in lung and colon as well as activated PBMC (Imai, et al. (1996)) while TECK was absent from these tissues. Besides the thymus, numerous reports indicate that T cell development can occur in the small intestine (Poussier and Julius (1994) Annu. Rev. Immunol. 12:521-
15 553) where TECK is also expressed. Interestingly, the liver has also been suggested to support T-cell development to some extent (Abo, et al. (1994) Int. Rev. Immunol. 11:61-102) and we observed a low TECK expression in a liver cDNA library. These data show that TECK
20 expression correlates with organs that support T-cell development.

While many molecular and cellular aspects of T-cell differentiation are well documented, the precise role of chemokines in T-cell development is still unknown.

25 Recently, it has been shown that the bone marrow stroma-derived CXC chemokine SDF-1 is important for B lymphopoiesis and myelopoiesis since SDF-1 $-/-$ mice are impaired for these functions (Nagasaki, et al. (1996)). Similarly, it is likely that chemokines act at different
30 steps of T-cell differentiation. Chemokines, together with the expression of appropriate adhesion molecules, may dictate the migration of uncommitted progenitors from the bone marrow to other anatomic locations. Indeed SDF-1 is chemoattractant for human CD34+ progenitor cells.
35 Aiuti, et al. (1997) J. Exp. Med. 185:111-120. The observation that TECK is chemoattractant for thymocytes but not for mature peripheral T cells suggests that TECK could attract T-cell progenitors to the thymus. Such populations are very difficult to isolate in sufficient

numbers to conduct in vitro chemotaxis experiments, but we are currently designing new strategies to address this important question. In addition, we have not found significant chemotactic activity of TECK on bone marrow 5 cells. SDF-1 was shown to be much less potent on CD34+ progenitors from the peripheral blood than those from the bone marrow. Aiuti, et al. (1997). It is possible that the sensitivity of progenitor cells to TECK would increase as these cells leave the bone marrow to colonize 10 lymphoid organs. Importantly, intra-thymic maturation is also characterized by a directional migration from the subcapsular region which contains the earliest progenitors to the cortex and finally to the medulla where thymocytes finish their maturation (Boyd, et al. 15 (1993)). It is possible that the secretion of TECK by medullary dendritic cells may play a role in this directional migration. Yet another possibility is that TECK may play a role in the organization and development 20 of the thymic stroma.

20 We also showed that TECK is chemotactic for activated macrophages and dendritic cells. These two cell types also play important roles in T-cell development. Through a complex screening process involving positive and negative selection events most of 25 the antigenic specificities randomly generated in the thymus will be eliminated by programmed cell death (Janeway (1994) Immunity 1:3-6). The efficient scavenging of dead thymocytes is probably mediated, at least partially, by thymic macrophages and thus TECK 30 could play an important role through its action on activated macrophages. Further along, T-cells with a high affinity for self-antigens and thus potentially harmful are eliminated through negative selection (Janeway (1994)). It is believed that thymic dendritic 35 cells are primarily responsible for the negative selection of thymocytes, therefore playing a major role in the establishment of tolerance. Inaba, et al. (1991) J. Exp. Med. 173:549-559. An efficient mechanism of central tolerance should eliminate T cells potentially

reactive against auto-antigens which are not expressed in the thymus, such as organ specific auto-antigens. Several known chemokines induce the migration of dendritic cells and could therefore contribute to their recruitment during peripheral immune responses. Sozzani, et al. (1995) J. Immunol. 155:3292-3295; and Xu, et al. (1996) J. Leukoc. Biol. 60:365-371. Similarly, dendritic cells presenting organ-specific or other antigens could be recruited to the thymus or the small intestine and induce negative selection of T cells specific for these antigens. It is possible that thymus- and small intestine-specific chemokines active on dendritic cells such as TECK could play an important role in the establishment of tolerance. Thus, TECK could potentially interact at several important steps of T-cell development. Future experiments will aim to define the precise role of TECK in T-cell development and other physiological processes through the use of genetically modified mice.

20 I. TECK is specifically expressed by thymic dendritic cells

Dendritic cells represent a heterogeneous cell population derived from bone marrow progenitors. They are present in non-lymphoid organs as immature dendritic cells (such as Langerhans cells in the skin) where they display a high ability for antigen capture. Cella, et al. (1997) Curr. Opin. Immunol. 9:10-16. Subsequent to antigen challenge, they will migrate to secondary lymphoid organs and will acquire a high capacity to present processed antigens to naive T-cells to initiate a specific immune response (Cella, et al. (1997)). It has been shown that dendritic cells can derive from CD34+ progenitors cultured in the presence of GM-CSF and TNF- α (Caux, et al. (1992) Nature 360:258-261; and Caux, et al. (1996) J. Exp. Med. 184:695-706) or from monocytes in the presence of GM-CSF and IL-4 (Sallusto and Lanzavecchia (1994) J. Exp. Med. 179:1109-1118). Interestingly, there is also evidence for a lymphoid dendritic cell precursor in thymus and bone marrow which is able to derive both

lymphocytes and dendritic cells in the absence of GM-CSF. Ardavin, et al. (1993) Nature 362:761-763; Galy, et al. (1995) Immunity 3:459-473; Marquez, et al. (1995) J. Exp. Med. 181:475-483; and Wu, et al. (1996). These lymphoid-derived dendritic cells may have different functional properties such as a negative regulation of T-cell responses since they express FasL in the mouse. Suss and Shortman (1996) J. Exp. Med. 183:1789-1796. We found that TECK was expressed at high levels in mouse thymic dendritic cells but was absent in cDNA libraries from mouse splenic dendritic cells or from human dendritic cells generated in vitro from CD34+ precursors or monocytes. Interestingly, mTECK mRNA was present at a low level in a population of early thymocyte progenitors still able to derive dendritic cells (Wu, et al. (1996)). Thus, it would be tempting to suggest that TECK could be a specific marker of lymphoid-derived dendritic cells. However, we observed that TECK was absent from splenic dendritic cells that likely contain lymphoid-derived dendritic cells. The expression of TECK mRNA appeared in the spleen of mice injected with LPS would suggest that peripheral dendritic cells may express TECK upon activation, but we found that TECK was not expressed in cDNA libraries of bone-marrow derived dendritic cells activated with LPS, PMA and ionomycin or IL-1 α and TNF- α . It is possible that the normal expression of TECK is specific for lymphoid-derived dendritic cells or, alternatively, that it is upregulated by very specific stimuli present in the thymic and intestinal micro-environment under physiological conditions. Consistent with the latter hypothesis is our observation of specific staining of thymic endothelial cells with anti-TECK antibody since we have not been able to find TECK expression in human HUVEC endothelial cells by northern blot analysis, without activation or following a 16 hour-activation with various combinations of IL-1, TNF- α , IL-4, IL-7 and oncostatin while some of these stimuli induce the expression of other CC chemokines in endothelial cells. Rollins and Pober (1991) Am. J. Pathol. 138:1315-

1319; Marfaing-Koka, et al. (1995) *J. Immunol.* 154:1870-1878; Garcia-Zepeda, et al. (1996) *J. Immunol.* 157:5613-5626; and Garcia-Zepeda, et al. (1996) *Nat. Med.* 4:449-456. Taken together, our data strongly suggest that TECK 5 is a novel chemokine specifically expressed by activated lymphoid-derived dendritic cells.

Through their function of antigen presentation, dendritic cells play major roles in the establishment of tolerance and in the initiation of an antigen-specific 10 immune response. The use of purified dendritic cells has been recently proposed in different therapeutic protocols (Cella, et al. (1997)). The discovery of factors with a regulated expression in dendritic cells such as the novel CC chemokine TECK will certainly improve our knowledge of 15 the biology of dendritic cells and lead to the design of relevant in vivo applications.

J. Mice and in vivo experimental procedures

Four to eight week-old and time-pregnant BALB/c mice were purchased from Simonsen Laboratories (Gilroy, CA). 20 RAG-1-deficient mice (Mombaerts, et al. (1992)) were purchased from The Jackson Laboratory (Bar Harbor, ME). To analyze TECK expression after in vivo activation, various organs were recovered from pools of 2 mice 3 hours after intravenous LPS injection (50 µg LPS in 200 25 µl PBS or 200 µl PBS for controls).

K. Cell purification, culture and stimulation.

THP-1 cells (TIB-202 from the American Type Culture Collection, Rockville, MD) were cultured in complete medium which consisted in RPMI 1640 medium (JRH 30 BioSciences, Lenexa, KS) supplemented with 10% FCS, 200 mM L-glutamin, 5 x 10⁻⁵ M mercaptoethanol, MEM amino-acids and vitamins, sodium bicarbonate, penicillin, streptomycin (all from Sigma, ST. Louis, MO), and gentamycin (Boehringer, Indianapolis, IN). To obtain 35 activated mouse macrophages, 10 ml of cold PBS were injected into the peritoneum and the collected cells allowed to adhere to plastic for 24 h in complete medium. The adherent fraction, mostly macrophages, was then collected. To obtain splenic dendritic cells, a

splenocyte cell suspension was prepared in RPMI 1640 Dutch modified medium (Life Technologies, Paisley, Scotland) as described previously in, e.g., Macatonia, et al. (1987) J. Exp. Med. 166:1654-1667. Splenocytes were 5 incubated at 37° C for 16 h and the cell suspension was collected and laid over Metrizamide (Nycomed Pharma, Oslo, Norway). After centrifugation for 10 min. at 1700 x g, the low interface was collected and stained with anti-Mac-1 (Pharmingen, San Diego, CA) and the anti-CD11c 10 N-418 antibodies (Macatonia, et al. (1993) J. Immunol. 150:3755-3765). Splenic dendritic cells were sorted by flow cytometry on a FACStar plus cell sorter (Becton Dickinson, Mountain View, CA) to a purity greater than 98% upon reanalysis in all the experiments included in 15 this report. To obtain thymic dendritic cells, thymuses were cut in small fragments and resuspended in 10 ml of RPMI-1640 +10% FCS containing 1 mg/ml collagenase and 0.02 mg/ml DNase I (both from SIGMA) and digested with continuous agitation at room temperature for 30 min. 20 (Shortman, et al. (1995) Adv. Exp. Med. Biol. 378:21-29). One ml of 0.1M EDTA pH 7.2 was added for an additional 5 min. Cells were then washed in complete medium, resuspended in complete medium and overlaid onto Metrizamide. The thymic dendritic cell-enriched 25 preparation was then stained with anti-IA_d and N-418 antibodies and the dendritic cells sorted by flow cytometry

L. Molecular cloning of mouse and human TECK

The cDNA encoding mouse TECK was obtained by random 30 sequencing of a RAG-1 KO mouse thymic directional cDNA library. Briefly, mRNA was extracted using RNAzolTM B (Tel-Test, Friendswood, TX) and then oligotex-dT mRNA kit (Quiagen, Chatsworth, CA) following the manufacturer's instruction. A directional cDNA library was prepared 35 using the SuperscriptTM Plasmid System (Gibco-BRL, Grand Island, NY) and cloned into the pME18s plasmid vector. Sequencing was done using the TaQ DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA). To determine whether TECK was present in other

mammals including human, a Southern blot containing EcoRI digested genomic DNA from different species (Bios Laboratories, New Haven, CT) was hybridized with the full-length mouse TECK cDNA.

5 The cDNA encoding human TECK was found by screening of a small intestine cDNA library using the full-length mouse TECK cDNA as a probe following standard procedures.
M. Northern blot analysis of RNA and Southern blot analysis of cDNA libraries

10 All RNA's were isolated from tissues or cells using RNAzol™ B (Tel-Test) and analyzed after electrophoresis in a 1% formaldehyde-agarose gel (10 µg/lane). RNA's were then blotted onto a Hybond-N+ nylon membrane (Amersham, Arlington Heights, IL). Some northern blots 15 of mRNA were bought from Clontech (Palo Alto, CA). To analyze the expression of TECK in cDNA libraries (obtained from T. MacClanahan, DNAX), 10 µg of DNA were digested with the appropriate restriction enzymes to release their inserts and analyze by Southern blotting 20 onto nylon membranes. Northern blots and blots of cDNA libraries were hybridized for 16 hours at 65°C with a ³²P-labeled probe consisting in the full-length cDNA encoding for mouse or human TECK and then washed and exposed, according to standard protocols.

25 N. Inter specific mouse backcross mapping

Inter specific backcross progeny were generated by mating (C57Bl/6J x M. spretus) F1 females and C57Bl/6J males as described, e.g., in Copeland and Jenkins (1991) Trends Genet. 7:113-118. A total of 205 N₂ mice was used 30 to map the Teck locus. DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer and hybridization with the full-length mTECK cDNA probe were performed as described, e.g., in Jenkins, et al. (1982). Fragments of 7.5, 6.9, and 2.5 kb were 35 detected in HincII digested C57Bl/6J DNA and fragments of 8.8 and 5.4 kb were detected in HincII digested M. spretus DNA. The presence or absence of the 8.8 and 5.4 kb HincII M. spretus-specific fragments, which cosegregated, was followed in backcross mice. A

description of the probes and RFLPs for two of the loci linked to Teck including Insr has been reported previously, e.g., in Ceci, et al. (1990) Genomics 6:72-79. Recombination distances were calculated as described 5 (Green (1981) "Linkage, recombination and mapping" pp. 77-113 in Genetics and Probability in Animal Breeding Experiments, Oxford University Press, New York) using the computer program SPRETUS MADNESS.

0. Measurement of TECK mRNA expression by RT-PCR
10 RNA's from sorted thymic dendritic cells or fetal thymuses were prepared with the RNeasy total RNA kit (Qiagen, Chatsworth, CA), following the manufacturer's instructions. First strand cDNAs were generated by reverse transcription with a random hexamer in a 10 μ l reaction and 1 μ l of this reaction was used as a template 15 for PCR. TECK expression was compared to the expression of hypoxanthine-guanine phosphoribosyl transferase (HPRT). Primer sequences were as follows: TECK: 5' primer, 5'CCTTCAGGTATCTGGAGAGGAGATC3' (nucleotides 58-72 of SEQ ID NO: 1) and 3' primer, 5'CACGCTGTACTGTTGGGGTC3' (complement of nucleotides 447-468 of SEQ ID NO: 1), HPRT: 5' primer, 5'GTAATGATCAGTCAACGGGGAC3' (SEQ ID NO: 17) and 3' primer, 5'CCAGCAAGCTTGCACCTAACCA3' (SEQ ID NO: 18).
20 Samples were submitted to 25 cycles of amplification, each composed of 94° C for 1 min., 57° C for 30 s and 72° C for 2 min. PCR products were then separated by 25 electrophoresis in 2% agarose gels and stained with ethidium bromide.

30 P. In Situ Hybridization
Biotin-14-CTP labeled sense and antisense riboprobes were generated using a non radioactive RNA labeling system (Gibco, Gaithersburg, MD) and the plasmid PCRII (InVitrogen, Carlsbad, CA) containing a 400 base pair 35 TECK cDNA fragment inserted by PCR and TA cloning (InVitrogen). Paraffin-embedded tissues were cut in 3-5 μ m sections, mounted on slides, baked at 60° C for one hour, deparaffinized in xylene (Fisher Scientific, Pittsburgh, PA) and immersed in 100% ethanol. Sections

were then incubated for 10 min at 37° C in proteinase K solution (40 mg/ml) (Gibco) in PBS and rinsed for 2 min in PBS at room temperature before being refixed in 10% formalin (Fisher Scientific, Pittsburgh, PA) in PBS for 1
5 min. Next, the sections were dehydrated through graded solutions of ethanol and air dried. Hybridization was carried out using the Gibco in situ hybridization and detection system kit. Vanadyl ribonucleoside complex (Gibco) was added to the hybridization solution (39 mM
10 final). A 0.1 µg/ml concentration of each probe was used during an 18 h hybridization at 42° C. Post-hybridization washes used room temperature 0.2X SSC. Following detection and substrate visualization, the slides were counterstained with 1% nuclear red stain
15 (Sigma, St. Louis, MO).

Q. Immunohistochemistry

A polyclonal antibody specific of a synthetic decapeptide identical to the C-terminus part of murine TECK (Figure 1) was prepared in rabbits by Research
20 Genetics (Huntsville, AL). Normal rabbit serum from a pool of 50 different animals (Research Genetics) was used as a negative control. To study TECK protein expression in the mouse thymus, 6 µm thick cryostat sections were thaw mounted on organosilicone subbed slides (American
25 Histology Reagent Co., Stockton, CA.) and fixed in 3% formalin (Fisher Scientific, Springfield, NJ) in Hank's Balanced Salt Solution with 0.01M HEPES (HBSS-HEPES), pH 7.4, for 15 min at room temperature. The sections were sequentially blocked for endogenous biotin binding using
30 the Vector blocking kit (Vector Laboratories, Burlingame, CA) and for endogenous peroxidase activity with a 1% hydrogen peroxide, 0.2M sodium azide solution, in HBSS-HEPES with 0.1% saponin (staining buffer). Non-specific antibody binding sites were then blocked with 10% normal
35 goat serum (Sigma) in staining buffer. Sections prepared as above were first incubated for 18 h at 25°C with 1/500 dilution of polyclonal antibody or control rabbit serum in staining buffer. In the second step, the sections were incubated for 1 h at room temperature with biotin

labeled goat anti-rabbit IgG (2 µg/ml) (Vector Laboratories) in staining buffer and then for 30 min at room temperature with the Vectastain Elite ABC Kit (Vector Laboratories) in staining buffer. The sections 5 were then rinsed in HBSS-HEPES without saponin. Immunoenzyme tissue staining was revealed with 3, 3' - diaminobenzidine tetrahydrochloride (DAB) substrate (0.5 mg/ml) (Sigma) in 0.05M Tris, pH 7.4, containing 0.0075% hydrogen peroxide. The substrate reaction was stopped by 10 rinsing the sections in distilled water. The sections were then counterstained with Harris' hematoxylin (Shandon Lipshaw, Pittsburg, PA).

The expression of TECK mRNA in murine adult thymus was analyzed by in situ hybridization and revealed a 15 discrete positive non-lymphoid population within the thymus medulla. The expression of TECK protein was analyzed by using a polyclonal anti-serum made in a rabbit immunised with a peptide that consisted in the last 12 amino-acid of the murine TECK protein sequence. 20 This polyclonal antibody reacts with the murine TECK recombinant protein prepared at DNAX both in ELISA and western blot. The application of this anti-serum on mouse adult thymic sections confirmed the distribution pattern obtained by in situ hybridization: the cells 25 producing TECK are medullary stromal cells. The precise cell type producing TECK within the mouse thymus was identified, using the same anti-serum on sorted thymic subsets, as being the thymic dendritic cells.

R. Production of recombinant mouse TECK in Escherichia 30 coli and other chemokines

Mouse recombinant TECK was produced in E. coli as an N-terminal FLAG (DYKDDDDKL; SEQ ID NO: 19) fusion protein. Briefly, the fusion construct containing FLAG followed by the mTECK sequence minus the leader peptide 35 (see SEQ ID NO: 2) was obtained by PCR amplification of the TECK cDNA in order to flank the coding sequence with HindIII and EcoRI sites and subsequent ligation in the pFLAG.1 vector which contains the FLAG sequence and an OmpA signal sequence. Electro-competent UT 4400 E. coli

were transformed with the pFLAG.1-mTECK plasmid. The cells were grown in 2 x LB plus 50 µg/ml Ampicillin, induced at an OD. of 2.3 with 400 µM IPTG and harvested. The cell pellet was resuspended in cold lysis buffer (20 mM Tris pH 8, 2 mM EDTA, 20% sucrose, 0.1 mg/ml lysozyme, 100 µl Benzonase), homogenized and allowed to sit for 30 min. Then the same amount of a 1:4 dilution of cold lysis buffer without lysozyme was added for 10 more min. The solution was spun and the supernatant was filtered through a 0.2 µm membrane and then diluted 1:1 in 50 mM Tris pH 7.5. The diluted osmotic extract was submitted to chromatography on a Q-sepharose column equilibrated with 50 mM Tris pH 7.5 and eluted with a linear salt gradient. The fractions containing the recombinant protein were pooled. The fractions were then loaded onto a S-sepharose column equilibrated with 20 mM acetate pH 4.0. The column was eluted with a linear salt gradient and then with a 1.5M NaCl wash that contained the protein. Finally, the eluate was loaded onto a reverse phase column. The column was eluted with a linear gradient of 20% to 80% acetonitrile + 0.1% TFA. The concentration of the mTECK protein was estimated by Comassie blue staining and densitometric scanning of a 10% Nu-PAGE gel with lysozyme as a standard. The purity was estimated at 100% by sequencing of the N-terminus of the recombinant protein. Recombinant murine MIP-1 α (R&D Systems, Minneapolis, MN) and lymphotactin (Hedrick, et al. (1997) *J. Immunol.* 158:1533-1540) were used as controls.

30 S. Assay for chemotaxis

The in vitro migration of cells isolated as described above in response to TECK or other factors was assessed in a modified Boyden micro chamber (Neuroprobe, Cabin John, MD) as described previously (Kelner, et al. (1994)). Briefly, factor dilutions in DMEM medium (Gibco) were loaded in the lower compartment in duplicate and 10⁵ cells in a 50 µl volume of DMEM were loaded in the upper compartment. The two compartments were separated by a 5-µm or 8-µm pore size polycarbonate

filter (Nucleopore, Pleasanton, CA). After incubation at 37° C for 80 min (or 120 min for lymphocytes), the filters were fixed in methanol and stained with Fields A and B. Cell migrated on the other side of the membrane 5 were counted per five high-power fields (100 x) under microscope. The chemotactic index was calculated from the number of cells counted with the test sample divided by the number of cells counted with medium alone.

Northern blot analysis was performed of RNA from 10 different organs hybridized with the mTECK cDNA probe with or without in vivo LPS stimulation. Hybridizing bands corresponded to the predicted ≈1040 bp size for mTECK mRNA. Significant induction occurred in spleen (with virtually no background), and in thymus and small 15 intestine (both with higher background); no signal was detected in either condition for heart, lung, kidney, or liver.

mTECK mRNA expression was analysed in the mouse fetal thymus. RNA's from fetal thymic lobes were 20 extracted at day 14, 15, 16 and 17 of gestation. Positive RT-PCR signals were detected in each of day 14, 125, 16, and 17 samples.

mTECK mRNA expression in thymic dendritic cells was evaluated. A population enriched in thymic dendritic 25 cells was prepared from 15 pooled adult thymuses. >99% pure dendritic cells were then sorted by flow cytometry based on their MHC Class II+ N-418+ phenotype. mTECK mRNA was then analyzed by RT-PCR and a MHC class II+ N- 418- population sorted in the same experiment was used as 30 a negative control. The N418+ sample gave a positive signal, while the N418- sample did not.

Expression analysis was performed with hTECK mRNA in different Human Tissues and Cell Types. Southern blots of human cDNA libraries digested with the appropriate 35 restriction enzymes were hybridized with the hTECK cDNA probe. A major band hybridizing corresponding to the predicted length of hTECK mRNA (≈1040 bp) was observed with sometimes some other bands that may represent incomplete cDNAs. Positive signals were detected in

tonsil, fetal spleen, and fetal small intestine. No signal was detected in activated (with PMA and ionomycin for 12 h) NK cells, activated (anti-CD40 antibody and IL-4 for 6 and 12 h) splenocytes, $\gamma\delta$ T cells, activated (with anti-CD3 and PMA for 6, 12, and 24 h) PBMC, fetal testis, C+ (elutriated monocytes cultured with IFN- γ and IL-10) monocytes, C- monocytes, 70% pure dendritic cells (CD1 α + dendritic cell population obtained by expansion of CD34+ bone marrow cells with GM-CSF and TNF- α and resting), and DC3 (similar dendritic cell population stimulated with PMA and ionomycin for 1 and 6 h), DC5 (dendritic cells obtained by culturing peripheral blood monocytes in the presence of IL-4 and GM-CSF), U937 (premonocytic cell line), and CD1 α cell lines. Ras KO mouse cDNA again confirmed that the mouse and human genes crosshybridize.

Four independent lines of transgenic mice expressing TECK in the brain have been made. All animals had neurologic disorders. In addition, several of them suffered severe infections. The consequences of TECK could be a direct one on brain cells which nature remains to be identify. Alternatively, since TECK has been shown in vitro to have effects on macrophages and dendritic cells which are critical effectors of immune responses, the overproduction of TECK could lead to distant effects on these cells at sites of infection. These results suggest that the blockade of TECK production in vivo may help to resolve particular pathological processes, in particular infections.

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VII. Screening for receptor/ligand

Labeled reagent is useful for screening of an expression library made from a cell line which expresses a chemokine or receptor, as appropriate. Standard staining techniques are used to detect or sort intracellular or surface expressed ligand, or surface expressing transformed cells are screened by panning. Screening of intracellular expression is performed by

various staining or immunofluorescence procedures. See also, e.g., McMahan, et al. (1991) EMBO J. 10:2821-2832.

For example, on day 0, precoat 2-chamber permanox slides with 1 ml per chamber of fibronectin, 10 ng/ml in 5 PBS, for 30 min at room temperature. Rinse once with PBS. Then plate COS cells at 2-3 x 10⁵ cells per chamber in 1.5 ml of growth media. Incubate overnight at 37° C.

On day 1 for each sample, prepare 0.5 ml of a 10 solution of 66 µg/ml DEAE-dextran, 66 µM chloroquine, and 4 µg DNA in serum free DME. For each set, a positive control is prepared, e.g., of huIL-10-FLAG cDNA at 1 and 1/200 dilution, and a negative mock. Rinse cells with serum free DME. Add the DNA solution and incubate 5 hr at 37° C. Remove the medium and add 0.5 ml 10% DMSO in 15 DME for 2.5 min. Remove and wash once with DME. Add 1.5 ml growth medium and incubate overnight.

On day 2, change the medium. On days 3 or 4, the 20 cells are fixed and stained. Rinse the cells twice with Hank's Buffered Saline Solution (HBSS) and fix in 4% paraformaldehyde (PFA)/glucose for 5 min. Wash 3X with HBSS. The slides may be stored at -80° C after all liquid is removed. For each chamber, 0.5 ml incubations are performed as follows. Add HBSS/saponin(0.1%) with 32 µl/ml of 1M NaN₃ for 20 min. Cells are then washed with 25 HBSS/saponin 1X. Add antibody complex to cells and incubate for 30 min. Wash cells twice with HBSS/saponin. Add second antibody, e.g., Vector anti-mouse antibody, at 1/200 dilution, and incubate for 30 min. Prepare ELISA solution, e.g., Vector Elite ABC horseradish peroxidase 30 solution, and preincubate for 30 min. Use, e.g., 1 drop of solution A (avidin) and 1 drop solution B (biotin) per 2.5 ml HBSS/saponin. Wash cells twice with HBSS/saponin. Add ABC HRP solution and incubate for 30 min. Wash cells twice with HBSS, second wash for 2 min, which closes 35 cells. Then add Vector diaminobenzoic acid (DAB) for 5 to 10 min. Use 2 drops of buffer plus 4 drops DAB plus 2 drops of H₂O₂ per 5 ml of glass distilled water. Carefully remove chamber and rinse slide in water. Air

dry for a few minutes, then add 1 drop of Crystal Mount and a cover slip. Bake for 5 min at 85-90° C.

Alternatively, the binding compositions are used to affinity purify or sort out cells expressing the ligand 5 or receptor. See, e.g., Sambrook, et al. or Ausubel et al.

All references cited herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and 10 individually indicated to be incorporated by reference.



SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT:

10

- (A) NAME: Schering Corp.
- (B) STREET: 2000 Galloping Hill Road
- (C) CITY: Kenilworth
- (D) STATE: New Jersey
- (E) COUNTRY: USA
- (F) ZIP: 07033-0530
- (G) TELEPHONE: 908-298-2906
- (H) TELEFAX: 908-298-5388

15

20

(ii) TITLE OF INVENTION: MAMMALIAN CHEMOKINE REAGENTS

(iii) NUMBER OF SEQUENCES: 19

25

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: Apple Macintosh
- (C) OPERATING SYSTEM: Macintosh 7.1
- (D) SOFTWARE: Microsoft Word 5.1a

20

(v) CURRENT APPLICATION DATA:

30

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

35

(vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: US 08/675,814
- (B) FILING DATE: 05-JUL-1996

40

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

45

- (A) LENGTH: 1034 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

55

- (A) NAME/KEY: CDS
- (B) LOCATION: 94..525

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

55 AGGCTACAAG CAGGCACCAAG CTCTCAGGAC CAGAAAGGCA TTGGTGGCCC CCTTAAACCT

60

TCAGGTATCT GGAGAGGAGA TCTAACCTTC ACT ATG AAA CTG TGG CTT TTT GCC
Met Lys Leu Trp Leu Phe Ala

114

1 5

60

TGC CTG GTT GCC TGT TTT GTT GGG GCC TGG ATG CCG GTT GTC CAT GCC
Cys Leu Val Ala Cys Phe Val Gly Ala Trp Met Pro Val Val His Ala

162

10

15

20

CAA GGT GCC TTT GAA GAC TGC TGC CTG GGT TAC CAG CAC AGG ATC AAA Gln Gly Ala Phe Glu Asp Cys Cys Leu Gly Tyr Gln His Arg Ile Lys 25 30 35	210
5 TGG AAT GTT CTC CGG CAT GCT AGG AAT TAT CAC CAG CAG GAA GTG AGT Trp Asn Val Leu Arg His Ala Arg Asn Tyr His Gln Gln Glu Val Ser 40 45 50 55	258
10 GGA AGC TGC AAC CTA CGT GCT GTG AGA TTC TAC TTC CGC CAG AAA GTA Gly Ser Cys Asn Leu Arg Ala Val Arg Phe Tyr Phe Arg Gln Lys Val 60 65 70	306
15 GTG TGT GGG AAT CCA GAG GAC ATG AAT GTG AAG AGG GCG ATA AGA ATC Val Cys Gly Asn Pro Glu Asp Met Asn Val Lys Arg Ala Ile Arg Ile 75 80 85	354
20 TTG ACA GCT AGG AAA AGG CTA GTC CAC TGG AAG ACC GCC TCA GAC TCT Leu Thr Ala Arg Lys Arg Leu Val His Trp Lys Ser Ala Ser Asp Ser 90 95 100	402
25 CAG ACT GAA AGG AAG TCA AAC CAT ATG AAG TCC AAG GTG GAG AAC Gln Thr Glu Arg Lys Lys Ser Asn His Met Lys Ser Lys Val Glu Asn 105 110 115	450
30 CCC AAC AGT ACA AGC GTG AGG AGT GCC ACC CTA GGT CAT CCC AGG ATG Pro Asn Ser Thr Ser Val Arg Ser Ala Thr Leu Gly His Pro Arg Met 120 125 130 135	498
35 GTG ATG ATG CCC AGA AAG ACC AAC AAT TAAGTTAATT ACTCAGAGTA Val Met Met Pro Arg Lys Thr Asn Asn 140	545
40 AGCACCCAGCT GGAGGGATCGG CGGAGTCTGC TGAAGTGCTG TCTTCTAGGC ATGCCAGTGC CAATGAACTC ACTGAAGCTA CAGTTTCTTG TACAAGACCA GACCCACCAA CGTCTCAGCA TGTACGGAGGA AGGAACACTACT GCGCTAAAGG CCCTCCCACT CACCAAGGAG CTATTGGCTA TTGATGATTG CTGAGGGAGG GGAGTAATT TTTTCTCTT TCTGAAGTGT GACTTGAGTA AATTGCCCAT AGTTCACTAT ATAATCCCCA ACCTGTGCTC AGGCAAGCAA CCCTAATTAA ATGCAATAGC CACATACAAA AGAAGAGGAT ATGAATAGTT TGGTAGGAGG GGCTTGTTAG 45 GAAGAAGACA TTAACAGGAG AGAGAGGAGC GAGAGGATAG TGAGTGTGTG AGAGTGCCTG CACGTGTGAA ATGGTCAAAG AATTAAAAAA TAAAAACTTA AAAAGCTATT AAAAAGTAAA 50 AAAAAATAAA	605 665 725 785 845 905 965 1025 1034

(2) INFORMATION FOR SEQ ID NO:2:

55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 144 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Leu Trp Leu Phe Ala Cys Leu Val Ala Cys Phe Val Gly Ala	
1 5 10 15	

Trp	Met	Pro	Val	Val	His	Ala	Gln	Gly	Ala	Phe	Glu	Asp	Cys	Cys	Leu				
														25	30				
5	Gly	Tyr	Gln	His	Arg	Ile	Lys	Trp	Asn	Val	Leu	Arg	His	Ala	Arg	Asn			
															35	40	45		
																50	55	60	
10	Phe	Tyr	Phe	Arg	Gln	Lys	Val	Val	Cys	Gly	Asn	Pro	Glu	Asp	Met	Asn			
																65	70	75	80
15	Val	Lys	Arg	Ala	Ile	Arg	Ile	Leu	Thr	Ala	Arg	Lys	Arg	Leu	Val	His			
																85	90	95	
	Trp	Lys	Ser	Ala	Ser	Asp	Ser	Gln	Thr	Glu	Arg	Lys	Lys	Ser	Asn	His			
																100	105	110	
20	Met	Lys	Ser	Lys	Val	Glu	Asn	Pro	Asn	Ser	Thr	Ser	Val	Arg	Ser	Ala			
																115	120	125	
	Thr	Leu	Gly	His	Pro	Arg	Met	Val	Met	Met	Pro	Arg	Lys	Thr	Asn	Asn			
																130	135	140	
25	(2) INFORMATION FOR SEQ ID NO:3:																		
	(i) SEQUENCE CHARACTERISTICS:																		
30	(A)	LENGTH:	1012	base	pairs														
	(B)	TYPE:	nucleic acid																
	(C)	STRANDEDNESS:	single																
	(D)	TOPOLOGY:	linear																
35	(ii) MOLECULE TYPE: cDNA																		
	(ix) FEATURE:																		
40	(A)	NAME/KEY:	CDS																
	(B)	LOCATION:	117..566																
	(ix) FEATURE:																		
45	(A)	NAME/KEY:	mat_peptide																
	(B)	LOCATION:	186..566																
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:																		
50	TCGACCCACG	CGTCCGCTTG	GCCTACAGCC	CGGCGGGCAT	CAGCTCCCTT	GACCCAGTGG										60			
	ATATCGGTGG	CCCCGTTATT	CGTCCAGGTG	CCCAAGGGAGG	AGGACCCGCC	TGCAGC										116			
	ATG	AAC	CTG	TGG	CTC	CTG	GCC	TGC	CTG	GCC	GGC	TTC	CTG	GGA	GCC	164			
	Met	Asn	Leu	Trp	Leu	Leu	Ala	Cys	Leu	Val	Ala	Gly	Leu	Gly	Ala				
55	-23	-20	-15												-10				
	TGG	GCC	CCC	GCT	GTC	CAC	ACC	CAA	GGT	GTC	TTT	GAG	GAC	TGC	CTG	212			
	Trp	Ala	Pro	Ala	Val	His	Thr	Gln	Gly	Val	Phe	Glu	Asp	Cys	Cys	Leu			
	-5																		
60	GCC	TAC	CAC	TAC	CCC	ATT	GGG	TGG	GCT	CTG	CGG	CGC	GCC	TGG	ACT	260			
	Ala	Tyr	His	Tyr	Pro	Ile	Gly	Trp	Ala	Val	Leu	Arg	Arg	Ala	Trp	Thr			
	10					15				20					25				

TAC CGG ATC CAG GAG GTG AGC GGG AGC TGC AAT CTG CCT GCT GCG ATA Tyr Arg Ile Gln Glu Val Ser Gly Ser Cys Asn Leu Pro Ala Ala Ile 30 35 40	308
5 TTC TAC CTC CCC AAG AGA CAC AGG AAG GTG TGT GGG AAC CCC AAA AGC Phe Tyr Leu Pro Lys Arg His Arg Lys Val Cys Gly Asn Pro Lys Ser 45 50 55	356
10 AGG GAG GTG CAG AGA GCC ATG AAG CTC CTG GAT GCT CGA AAT AAG GTT Arg Glu Val Gln Arg Ala Met Lys Leu Leu Asp Ala Arg Asn Lys Val 60 65 70	404
15 TTT GCA AAG CTC CAC CAC AAC ATG CAG ACC TTC CAA GCA GGC CCT CAT Phe Ala Lys Leu His His Asn Met Gln Thr Phe Gln Ala Gly Pro His 75 80 85	452
20 GCT GTA AAG AAG TTG AGT TCT GGA AAC TCC AAG TTA TCA TCA TCC AAG Ala Val Lys Lys Leu Ser Ser Gly Asn Ser Lys Leu Ser Ser Ser Lys 90 95 100 105	500
25 TTT AGC AAT CCC ATC AGC AGC AAG AGG AAT GTC TCC CTC CTG ATA Phe Ser Asn Pro Ile Ser Ser Lys Arg Asn Val Ser Leu Leu Ile 110 115 120	548
30 TCA GCT AAT TCA GGA CTG TGAGCCGGCT CATTCTGGG CTCCATCGGC Ser Ala Asn Ser Gly Leu 125	596
35 ACAGGAGGGG CCGGATCTTT CTCCGATAAA ACCGTCGCCCTACAGACCCA GCTGTCCCCA CGCCTCTGTC TTTGGGTCA AGTCTTAATC CCTGCACCTG AGTTGGTCCT CCCTCTGCAC CCCCACCACC TCCTGCCCGT CTGGCAACTG GAAAGAAGGA GTTGGCCTGA TTTAACCTT	656
40 TTGCCGCTCC GGGGAACAGC ACAATCCTGG GCAGCCAGTG GCTCTTGTAG AGAAAACCTTA GGATACCTCT CTCACTTTCT GTTTCTTGCC GTCCACCCCG GGCCATGCCA GTGTGTCTC TGGGTCCCTT CCAAAATCT GGTCAATTCAA GGATCCCCTC CCAAGGCTAT GCTTTCTAT AACTTTAAA TAAACCTTGG GGGGTGAATG GAATAAAAAA AAAAAAAA AAAAAA	716
45 AACTTTAAA TAAACCTTGG GGGGTGAATG GAATAAAAAA AAAAAAAA AAAAAA 1012	776

(2) INFORMATION FOR SEQ ID NO:4:
45

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 150 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

55 Met Asn Leu Trp Leu Leu Ala Cys Leu Val Ala Gly Phe Leu Gly Ala -23 -20 -15 -10
60 Trp Ala Pro Ala Val His Thr Gln Gly Val Phe Glu Asp Cys Cys Leu -5 1 5
65 Ala Tyr His Tyr Pro Ile Gly Trp Ala Val Leu Arg Arg Ala Trp Thr 10 15 20 25

Tyr Arg Ile Gln Glu Val Ser Gly Ser Cys Asn Leu Pro Ala Ala Ile
 30 35 40
 Phe Tyr Leu Pro Lys Arg His Arg Lys Val Cys Gly Asn Pro Lys Ser
 5 45 50 55
 Arg Glu Val Gln Arg Ala Met Lys Leu Leu Asp Ala Arg Asn Lys Val
 60 65 70
 10 Phe Ala Lys Leu His His Asn Met Gln Thr Phe Gln Ala Gly Pro His
 75 80 85
 Ala Val Lys Lys Leu Ser Ser Gly Asn Ser Lys Leu Ser Ser Ser Lys
 90 95 100 105
 15 Phe Ser Asn Pro Ile Ser Ser Ser Lys Arg Asn Val Ser Leu Leu Ile
 110 115 120
 Ser Ala Asn Ser Gly Leu
 20 125

(2) INFORMATION FOR SEQ ID NO:5:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 801 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

35 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..288

40 (ix) FEATURE:
 (A) NAME/KEY: mat_peptide
 (B) LOCATION: 79..288

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

45	ATG TGC TGT ACC AAG AGT TTG CTC CTG GCT GCT TTG ATG TCA GTG CTG Met Cys Cys Thr Lys Ser Leu Leu Leu Ala Ala Leu Met Ser Val Leu -26 -25 -20 -15	48
50	CTA CTC CAC CTC TGC GGC GAA TCA GAA GCA GCA AGC AAC TTT GAC TGC Leu Leu His Leu Cys Gly Glu Ser Glu Ala Ala Ser Asn Phe Asp Cys -10 -5 1 5	96
55	TGT CTT GGA TAC ACA GAC CGT ATT CTT CAT CCT AAA TTT ATT GTG GGC Cys Leu Gly Tyr Thr Asp Arg Ile Leu His Pro Lys Phe Ile Val Gly 10 15 - 20	144
60	TTC ACA CGG CAG CTG GCC AAT GAA GGC TGT GAC ATC AAT GCT ATC ATC Phe Thr Arg Gln Leu Ala Asn Glu Gly Cys Asp Ile Asn Ala Ile Ile 25 30 35	192
60	TTT CAC ACA AAG AAA AAG TTG TCT GTG TGC GCA AAT CCA AAA CAG ACT Phe His Thr Lys Lys Leu Ser Val Cys Ala Asn Pro Lys Gln Thr 40 45 50	240

TGG	GTG	AAA	TAT	ATT	GTG	CGT	CTC	CTC	AGT	AAA	GTC	AAG	AAC	ATG	288
Trp	Val	Lys	Tyr	Ile	Val	Arg	Leu	Leu	Ser	Lys	Lys	Val	Lys	Asn	Met
55				60					65				70		
5	TAAAAAACTGT	GGCTTTCTG	GAATGGAATT	GGACATAGCC	CAAGAACAGA	AAGAACCTTG	348								
	CTGGGGTTGG	AGGTTTCACT	TGCACATCAT	GGAGGGTTTA	GTGCTTATCT	AATTTGTGCC	408								
10	TCACTGGACT	TGTCCAATTA	ATGAAGTTGA	TTCATATTGC	ATCATAGTTT	GCTTTGTTTA	468								
	AGCATCACAT	TAAAGTTAAA	CTGTATTTTA	TGTTATTTAT	AGCTGTAGGT	TTTCTGTGTT	528								
15	TAGCTATTAA	ATACTAATT	TCCATAAGCT	ATTTGGTTT	AGTGCAAAGT	ATAAAATTAT	588								
	ATTTGGGGGG	GAATAAGATT	ATATGGACTT	TTTGCAAGC	AACAAGCTAT	TTTTAAAAAA	648								
	AAACTATTAA	ACATTCTTTT	GTTTATATTG	TTTGTCTCC	TAAATTGTTG	TAATTGCATT	708								
20	ATAAAATAAG	AAAAATATTA	ATAAGACAAA	TATTGAAAAT	AAAGAAACAA	AAAGTTAAAA	768								
	AAAAAAA	AAAAAAA	AAAAAAA	AAAAAAA	AAA		801								

(2) INFORMATION FOR SEQ ID NO:6:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 96 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

35	Met	Cys	Cys	Thr	Lys	Ser	Leu	Leu	Leu	Ala	Ala	Leu	Met	Ser	Val	Leu
	-26	-25					-20					-15				
	Leu	Leu	His	Leu	Cys	Gly	Glu	Ser	Glu	Ala	Ala	Ser	Asn	Phe	Asp	Cys
40	-10				-5					1				5		
	Cys	Leu	Gly	Tyr	Thr	Asp	Arg	Ile	Leu	His	Pro	Lys	Phe	Ile	Val	Gly
	10				15					20						
45	Phe	Thr	Arg	Gln	Leu	Ala	Asn	Glu	Gly	Cys	Asp	Ile	Asn	Ala	Ile	Ile
	25			30				35								
	Phe	His	Thr	Lys	Lys	Leu	Ser	Val	Cys	Ala	Asn	Pro	Lys	Gln	Thr	
50	40			45				50								
	Trp	Val	Lys	Tyr	Ile	Val	Arg	Leu	Leu	Ser	Lys	Lys	Val	Lys	Asn	Met
	55			60				65			70					

(2) INFORMATION FOR SEQ ID NO:7:

55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 699 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 142..435

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

10	GGCACGAGCG GCACGAGCAT CACTCACACC TTGCATTTCA CCCCTGCATC CCAGTCGCC	60
15	TGCAGCCTCA CACAGATCCT GCACACACCC AGACAGCTGG CGCTCACACA TTCACCGTTG	120
	GCCTGCCTCT GTTCACCCCTC C ATG GCC CTG CTA CTG GCC CTC AGC CTG CTG	171
	Met Ala Leu Leu Leu Ala Leu Ser Leu Leu	
	1 5 10	
20	GTT CTC TGG ACT TCC CCA GCC CCA ACT CTG AGT GGC ACC AAT GAT GCT	219
	Val Leu Trp Thr Ser Pro Ala Pro Thr Leu Ser Gly Thr Asn Asp Ala	
	15 20 25	
25	GAA GAC TGC TGC CTG TCT GTG ACC CAG AAA CCC ATC CCT GGG TAC ATC	267
	Glu Asp Cys Cys Leu Ser Val Thr Gln Lys Pro Ile Pro Gly Tyr Ile	
	30 35 40	
30	GTG AGG AAC TTC CAC TAC CTT CTC ATC AAG GAT GGC TGC AGG GTG CCT	315
	Val Arg Asn Phe His Tyr Leu Leu Lys Asp Gly Cys Arg Val Pro	
	45 50 55	
35	GCT GTA GTG TTC ACC ACA CTG AGG GGC CGC CAG CTC TGT GCA CCC CCA	363
	Ala Val Val Phe Thr Thr Leu Arg Gly Arg Gln Leu Cys Ala Pro Pro	
	60 65 70	
40	GAC CAG CCC TGG GTA GAA CGC ATC ATC CAG AGA CTG CAG AGG ACC TCA	411
	Asp Gln Pro Trp Val Glu Arg Ile Ile Gln Arg Leu Gln Arg Thr Ser	
	75 80 85 90	
45	GCC AAG ATG AAG CGC CGC AGC AGT TAACCTATGA CCGTGCAGAG GGAGCCCGGA	465
	Ala Lys Met Lys Arg Arg Ser Ser	
	95	
50	GTCCGAGTCA AGCATTGTGA ATTATTACCT AACCTGGGA ACCGAGGACC AGAAGGAAGG	525
	ACCAAGGCTTC CAGCTCCTCT GCACCCAGACC TGACCCAGCA GGACAGGGCC TGGGGTGTGT	585
55	GTGAGTGTGA GTGTGAGCGA GAGGGTGAGT GTGGTCTAGA GTAAAGCTGC TCCACCCCCA	645
	GATTCCAATG CTACCAATAA AGCCGCCTGG TGTTTACAAC TAAAAAAA AAAA	699

(2) INFORMATION FOR SEQ ID NO:8:

50

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 amino acids
- (B) TYPE: amino acid
- (C) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

60	Met Ala Leu Leu Leu Ala Leu Ser Leu Leu Val Leu Trp Thr Ser Pro	
	1 5 10 15	
	Ala Pro Thr Leu Ser Gly Thr Asn Asp Ala Glu Asp Cys Cys Leu Ser	
	20 25 30	

Val Thr Gln Lys Pro Ile Pro Gly Tyr Ile Val Arg Asn Phe His Tyr
 35 40 45
 5 Leu Leu Ile Lys Asp Gly Cys Arg Val Pro Ala Val Val Phe Thr Thr
 50 55 60
 Leu Arg Gly Arg Gln Leu Cys Ala Pro Pro Asp Gln Pro Trp Val Glu
 65 70 75 80
 10 Arg Ile Ile Gln Arg Leu Gln Arg Thr Ser Ala Lys Met Lys Arg Arg
 85 90 95
 Ser Ser
 15

(2) INFORMATION FOR SEQ ID NO:9:

20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1119 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA

30 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..1095
 (D) OTHER INFORMATION: Nucleotide 579 may be A, C, G
 or T, and the codon may code for His or Gln.

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATG TTT TCG ACT CCA GTG AAG ATT ATT TTG TGT CAG TCA ATA CTT CAT	48
Met Phe Ser Thr Pro Val Lys Ile Ile Leu Cys Gln Ser Ile Leu His	
40 1 5 10 15	
ATT ACT CAG TTG ATT CTG AGA TGT TAC TGT GCT CCT TGC AGG AGG TCA	96
Ile Thr Gln Leu Ile Leu Arg Cys Tyr Cys Ala Pro Cys Arg Arg Ser	
45 20 25 30	
GGC AGT TCT CCA GGC TAT TTG TAC CGA ATT GCC TAC TCC TTG ATC TGT	144
Gly Ser Ser Pro Gly Tyr Leu Tyr Arg Ile Ala Tyr Ser Leu Ile Cys	
50 35 40 45	
GTT CTT GGC CTC CTG GGG AAT ATT CTG GTG GTG ATC ACC TTT GCT TTT	192
Val Leu Gly Leu Leu Gly Asn Ile Leu Val Val Ile Thr Phe Ala Phe	
55 50 55 60	
TAT AAG AAG GCC AGG TCT ATG ACA GAC GTC TAT CTC TTG AAC ATG GCC	240
Tyr Lys Lys Ala Arg Ser Met Thr Asp Val Tyr Leu Leu Asn Met Ala	
65 65 70 75 80	
ATT GCA GAC ATC CTC TTT GTT CTT ACT CTC CCA TTC TGG GCA GTG AGT	288
Ile Ala Asp Ile Leu Phe Val Leu Thr Leu Pro Phe Trp Ala Val Ser	
60 85 90 95	
CAT GCC ACT GGT GCG TGG GTT TTC AGC AAT GCC ACG TGC AAG TTG CTA	336
His Ala Thr Gly Ala Trp Val Phe Ser Asn Ala Thr Cys Lys Leu Leu	
100 105 110	

	AAA GGC ATC TAT GCC ATC AAC TTT AAC TGC GGG ATG CTG CTC CTG ACT Lys Gly Ile Tyr Ala Ile Asn Phe Asn Cys Gly Met Leu Leu Leu Thr 115 120 125	384
5	TGC ATT AGC ATG GAC CGG TAC ATC GCC ATT GTA CAG GCG ACT AAG TCA Cys Ile Ser Met Asp Arg Tyr Ile Ala Ile Val Gln Ala Thr Lys Ser 130 135 140	432
10	TTC CGG CTC CGA TCC AGA ACA CTA CCG CGC AGC AAA ATC ATC TGC CTT Phe Arg Leu Arg Ser Arg Thr Leu Pro Arg Ser Lys Ile Ile Cys Leu 145 150 155 160	480
15	GTT GTG TGG GGG CTG TCA GTC ATC ATC TCC AGC TCA ACT TTT GTC TTC Val Val Trp Gly Leu Ser Val Ile Ile Ser Ser Ser Thr Phe Val Phe 165 170 175	528
20	AAC CAA AAA TAC AAC ACC CAA GGC AGC GAT GTC TGT GAA CCC AAG TAC Asn Gln Lys Tyr Asn Thr Gln Gly Ser Asp Val Cys Glu Pro Lys Tyr 180 185 190	576
	CAN ACT GTC TCG GAG CCC ATC AGG TGG AAG CTG CTG ATG TTG GGG CTT Xaa Thr Val Ser Glu Pro Ile Arg Trp Lys Leu Leu Met Leu Gly Leu 195 200 205	624
25	GAG CTA CTC TTT GGT TTC TTT ATC CCT TTG ATG TTC ATG ATA TTT TGT Glu Leu Leu Phe Gly Phe Ile Pro Leu Met Phe Met Ile Phe Cys 210 215 220	672
30	TAC ACG TTC ATT GTC AAA ACC TTG GTG CAA GCT CAG AAT TCT AAA AGG Tyr Thr Phe Ile Val Lys Thr Leu Val Gln Ala Gln Asn Ser Lys Arg 225 230 235 240	720
35	CAC AAA GCC ATC CGT GTA ATC ATA GCT GTG GTG CTT GTG TTT CTG GCT His Lys Ala Ile Arg Val Ile Ile Ala Val Val Leu Val Phe Leu Ala 245 250 255	768
40	TGT CAG ATT CCT CAT AAC ATG GTC CTG CTT GTG ACG GCT GCT AAT TTG Cys Gln Ile Pro His Asn Met Val Leu Leu Val Thr Ala Ala Asn Leu 260 265 270	816
	GGT AAA ATG AAC CGA TCC TGC CAG AGC GAA AAG CTA ATT GGC TAT ACG Gly Lys Met Asn Arg Ser Cys Gln Ser Glu Lys Leu Ile Gly Tyr Thr 275 280 285	864
45	AAA ACT GTC ACA GAA GTC CTG GCT TTC CTG CAC TGC TGC CTG AAC CCT Lys Thr Val Thr Glu Val Leu Ala Phe Leu His Cys Cys Leu Asn Pro 290 295 300	912
50	GTC CTC TAC GCT TTT ATT GGG CAG AAG TTC AGA AAC TAC TTT CTG AAG Val Leu Tyr Ala Phe Ile Gly Gln Lys Phe Arg Asn Tyr Phe Leu Lys 305 310 315 320	960
55	ATC TTG AAG GAC CTG TGG TGT GTG AGA AGG AAG TAC AAG TCC TCA GGC Ile Leu Lys Asp Leu Trp Cys Val Arg Arg Lys Tyr Lys Ser Ser Gly 325 330 335	1008
60	TTC TCC TGT GCC GGG AGG TAC TCA GAA AAC ATT TCT CCG CAG ACC AGT Phe Ser Cys Ala Gly Arg Tyr Ser Glu Asn Ile Ser Arg Gln Thr Ser 340 345 350	1056
	GAG ACC GCA GAT AAC GAC AAT GCG TCG TCC TTC ACT ATG TGATAGAAAG Glu Thr Ala Asp Asn Asp Asn Ala Ser Ser Phe Thr Met 355 360 365	1105

CTGAGTCTCC CTAA

1119

5 (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 365 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

15 (D) OTHER INFORMATION: Amino acid at position 193 may be His or Gln.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

20	Met	Phe	Ser	Thr	Pro	Val	Lys	Ile	Ile	Leu	Cys	Gln	Ser	Ile	Leu	His
	1				5				10					15		
	Ile Thr Gln Leu Ile Leu Arg Cys Tyr Cys Ala Pro Cys Arg Arg Ser															
		20			25					30						
25	Gly	Ser	Ser	Pro	Gly	Tyr	Leu	Tyr	Arg	Ile	Ala	Tyr	Ser	Leu	Ile	Cys
		35				40				45						
30	Val	Leu	Gly	Leu	Leu	Gly	Asn	Ile	Leu	Val	Val	Ile	Thr	Phe	Ala	Phe
		50			55				60							
	Tyr	Lys	Lys	Ala	Arg	Ser	Met	Thr	Asp	Val	Tyr	Leu	Leu	Asn	Met	Ala
	65				70			75		80						
35	Ile	Ala	Asp	Ile	Leu	Phe	Val	Leu	Thr	Leu	Pro	Phe	Trp	Ala	Val	Ser
			85			90			95							
	His	Ala	Thr	Gly	Ala	Trp	Val	Phe	Ser	Asn	Ala	Thr	Cys	Lys	Leu	Leu
	100			105						110						
40	Lys	Gly	Ile	Tyr	Ala	Ile	Asn	Phe	Asn	Cys	Gly	Met	Leu	Leu	Leu	Thr
	115			120			125									
45	Cys	Ile	Ser	Met	Asp	Arg	Tyr	Ile	Ala	Ile	Val	Gln	Ala	Thr	Lys	Ser
	130			135			140									
	Phe	Arg	Leu	Arg	Ser	Arg	Thr	Leu	Pro	Arg	Ser	Lys	Ile	Ile	Cys	Leu
	145			150			155			160						
50	Val	Val	Trp	Gly	Leu	Ser	Val	Ile	Ile	Ser	Ser	Ser	Thr	Phe	Val	Phe
			165			170			175							
	Asn	Gln	Lys	Tyr	Asn	Thr	Gln	Gly	Ser	Asp	Val	Cys	Glu	Pro	Lys	Tyr
	180			185						190						
55	Gln	Thr	Val	Ser	Glu	Pro	Ile	Arg	Trp	Lys	Leu	Leu	Met	Leu	Gly	Leu
	195			200			205									
60	Glu	Leu	Leu	Phe	Gly	Phe	Phe	Ile	Pro	Leu	Met	Phe	Met	Ile	Phe	Cys
	210			215			220									
	Tyr	Thr	Phe	Ile	Val	Lys	Thr	Leu	Val	Gln	Ala	Gln	Asn	Ser	Lys	Arg
	225			230			235			240						

	His Lys Ala Ile Arg Val Ile Ile Ala Val Val Leu Val Phe Leu Ala			
	245	250	255	
5	Cys Gln Ile Pro His Asn Met Val Leu Leu Val Thr Ala Ala Asn Leu			
	260	265	270	
	Gly Lys Met Asn Arg Ser Cys Gln Ser Glu Lys Leu Ile Gly Tyr Thr			
	275	280	285	
10	Lys Thr Val Thr Glu Val Leu Ala Phe Leu His Cys Cys Leu Asn Pro			
	290	295	300	
	Val Leu Tyr Ala Phe Ile Gly Gln Lys Phe Arg Asn Tyr Phe Leu Lys			
	305	310	315	320
15	Ile Leu Lys Asp Leu Trp Cys Val Arg Arg Lys Tyr Lys Ser Ser Gly			
	325	330	335	
20	Phe Ser Cys Ala Gly Arg Tyr Ser Glu Asn Ile Ser Arg Gln Thr Ser			
	340	345	350	
	Glu Thr Ala Asp Asn Asn Ala Ser Ser Phe Thr Met			
	355	360	365	

25 (2) INFORMATION FOR SEQ ID NO:11:

	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 1547 base pairs	
	(B) TYPE: nucleic acid	
30	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
35	(ix) FEATURE:	
	(A) NAME/KEY: CDS	
	(B) LOCATION: 49..1116	
40	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:	
	GAGGAAGCTG CTTCGGGGGG TGAGCAAACT TTTTAAATG CAGAAATT ATG ATC TAC	57
	Met Ile Tyr	
45	1	
	ACC CGT TTC TTA AAA GGC ACT CTG AAG ATG GCC AAT TAC ACG CTG GCA	105
	Thr Arg Phe Leu Lys Gly Ser Leu Lys Met Ala Asn Tyr Thr Leu Ala	
	5 10 15	
50	CCA GAG GAT GAA TAT GAT GTC CTC ATA GAA GGT GAA CTG GAG AGC GAT	153
	Pro Glu Asp Glu Tyr Asp Val Leu Ile Glu Gly Glu Leu Glu Ser Asp	
	20 25 30 35	
55	GAG GCA GAG CAA TGT GAC AAG TAT GAC GCC CAG GCA CTC TCA GCC CAG	201
	Glu Ala Glu Gln Cys Asp Lys Tyr Asp Ala Gln Ala Leu Ser Ala Gln	
	40 45 50	
60	CTG GTG CCA TCA CTC TGC TCT GCT GTG TTT GTG ATC GGT GTC CTG GAC	249
	Leu Val Pro Ser Leu Cys Ser Ala Val Phe Val Ile Gly Val Leu Asp	
	55 60 65	

	AAT CTC CTG GTT GTG CTT ATC CTG GTA AAA TAT AAA GGA CTC AAA CGC Asn Leu Leu Val Val Leu Ile Leu Val Lys Tyr Lys Gly Leu Lys Arg 70 75 80	297
5	GTG GAA AAT ATC TAT CTT CTA AAC TTG GCA GTT TCT AAC TTG TGT TTC Val Glu Asn Ile Tyr Leu Leu Asn Leu Ala Val Ser Asn Leu Cys Phe 85 90 95	345
10	TTG CTT ACC CTG CCC TTC TGG GCT CAT GCT GGG GGC GAT CCC ATG TGT Leu Leu Thr Leu Pro Phe Trp Ala His Ala Gly Gly Asp Pro Met Cys 100 105 110 115	393
15	AAA ATT CTC ATT GGA CTG TAC TTC GTG GGC CTG TAC AGT GAG ACA TTT Lys Ile Leu Ile Gly Leu Tyr Phe Val Gly Leu Tyr Ser Glu Thr Phe 120 125 130	441
20	TTC AAT TGC CTT CTG ACT GTG CAA AGG TAC CTA GTG TTT TTG CAC AAG Phe Asn Cys Leu Leu Thr Val Gln Arg Tyr Leu Val Phe Leu His Lys 135 140 145	489
25	GGC AAC TTT TTC TCA GCC AGG AGG GTG CCC TGT GGC ATC ATT ACA Gly Asn Phe Phe Ser Ala Arg Arg Arg Val Pro Cys Gly Ile Ile Thr 150 155 160	537
30	AGT GTC CTG GCA TGG GTA ACA GCC ATT CTG GCC ACT TTG CCT GAA TTC Ser Val Leu Ala Trp Val Thr Ala Ile Leu Ala Thr Leu Pro Glu Phe 165 170 175	585
35	GTG GTT TAT AAA CCT CAG ATG GAA GAC CAG AAA TAC AAG TGT GCA TTT Val Val Tyr Lys Pro Gln Met Glu Asp Gln Lys Tyr Lys Cys Ala Phe 180 185 190 195	633
40	AGC AGA ACT CCC TTC CTG CCA GCT GAT GAG ACA TTC TGG AAG CAT TTT Ser Arg Thr Pro Phe Leu Pro Ala Asp Glu Thr Phe Trp Lys His Phe 200 205 210	681
45	CTG ACT TTA AAA ATG AAC ATT TCG GTT CTT GTC CTC CCC CTA TTT ATT Leu Thr Leu Lys Met Asn Ile Ser Val Leu Val Leu Pro Leu Phe Ile 215 220 225	729
50	TTT ACA TTT CTC TAT GTG CAA ATG AGA AAA ACA CTA AGG TTC AGG GAG Phe Thr Phe Leu Tyr Val Gln Met Arg Lys Thr Leu Arg Phe Arg Glu 230 235 240	777
55	CAG AGG TAT AGC CTT TTC AAG CTT GTT TTT GCC GTA ATG GTA GTC TTC Gln Arg Tyr Ser Leu Phe Lys Leu Val Phe Ala Val Met Val Val Phe 245 250 255	825
60	CTT CTG ATG TGG GCG CCC TAC AAT ATT GCA TTT TTC CTG TCC ACT TTC Leu Leu Met Trp Ala Pro Tyr Asn Ile Ala Phe Phe Leu Ser Thr Phe 260 265 270 275	873
	AAA GAA CAC TTC TCC CTG AGT GAC TGC AAG AGC AGC TAC AAT CTG GAC Lys Glu His Phe Ser Leu Ser Asp Cys Lys Ser Ser Tyr Asn Leu Asp 280 285 290	921
	AAA AGT GTT CAC ATC ACT AAA CTC ATC GCC ACC ACC CAC TGC TGC ATC Lys Ser Val His Ile Thr Lys Leu Ile Ala Thr Thr His Cys Cys Ile 295 300 305	969
	AAC CCT CTC CTG TAT GCG TTT CTT GAT GGG ACA TTT AGC AAA TAC CTC Asn Pro Leu Leu Tyr Ala Phe Leu Asp Gly Thr Phe Ser Lys Tyr Leu 310 315 320	1017

	TGC CGC TGT TTC CAT CTG CGT AGT AAC ACC CCA CTT CAA CCC AGG GGG Cys Arg Cys Phe His Leu Arg Ser Asn Thr Pro Leu Gln Pro Arg Gly 325 330 335	1065
5	CAG TCT GCA CAA GGC ACA TCG AGG GAA GAA CCT GAC CAT TCC ACC GAA Gln Ser Ala Gln Gly Thr Ser Arg Glu Pro Asp His Ser Thr Glu 340 345 350 355	1113
10	G TG TAAACTAGCA TCCACCAAAT GCAAGAAGAA TAAACATGGA TTTTCATCTT Val	1166
	TCTGCATTAT TTCATGTAAA TTTTCTACAC ATTTGTATAC AAAATCGGAT ACAGGAAGAA	1226
15	AAGGGAGAGG TGAGCTAAC A TTTGCTAAC ACTGAATTG TCTCAGGCAC CGTGCAAGGC TCTTTACAAA CGTGAGCTCC TTGCTCCT ACCACTTGTG CATA GTGTGG ATAGGACTAG	1286
20	TCTCATTCT CTGAGAAGAA AACTAAGGCG CGGAAATTG TCTAAGATCA CATAACTAGG AAGTGGCAGA ACTGATTCTC CAGCCCTGGT AGCATTGCT CAGAGCCTAC GCTTGGTCCA	1346
	GAACATCAA CTCCAAACCC TGGGGACAAA CGACATGAAA TAAATGTATT TTAAACATA	1406
25	TAAAAAAA AAAAAAAA A	1466
		1526
		1547

(2) INFORMATION FOR SEQ ID NO:12:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 356 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

40	Met Ile Tyr Thr Arg Phe Leu Lys Gly Ser Leu Lys Met Ala Asn Tyr 1 5 10 15
	Thr Leu Ala Pro Glu Asp Glu Tyr Asp Val Leu Ile Glu Gly Glu Leu 20 25 30
45	Glu Ser Asp Glu Ala Glu Gln Cys Asp Lys Tyr Asp Ala Gln Ala Leu 35 40 45
	Ser Ala Gln Leu Val Pro Ser Leu Cys Ser Ala Val Phe Val Ile Gly 50 55 60
50	Val Leu Asp Asn Leu Leu Val Val Leu Ile Leu Val Lys Tyr Lys Gly 65 70 75 80
	Leu Lys Arg Val Glu Asn Ile Tyr Leu Leu Asn Leu Ala Val Ser Asn 55 85 90 95
	Leu Cys Phe Leu Leu Thr Leu Pro Phe Trp Ala His Ala Gly Gly Asp 100 105 110
60	Pro Met Cys Lys Ile Leu Ile Gly Leu Tyr Phe Val Gly Leu Tyr Ser 115 120 125
	Glu Thr Phe Phe Asn Cys Leu Leu Thr Val Gln Arg Tyr Leu Val Phe 130 135 140

Leu His Lys Gly Asn Phe Phe Ser Ala Arg Arg Arg Val Pro Cys Gly
 145 150 155 160

5 Ile Ile Thr Ser Val Leu Ala Trp Val Thr Ala Ile Leu Ala Thr Leu
 165 170 175

Pro Glu Phe Val Val Tyr Lys Pro Gln Met Glu Asp Gln Lys Tyr Lys
 180 185 190

10 Cys Ala Phe Ser Arg Thr Pro Phe Leu Pro Ala Asp Glu Thr Phe Trp
 195 200 205

Lys His Phe Leu Thr Leu Lys Met Asn Ile Ser Val Leu Val Leu Pro
 15 210 215 220

Leu Phe Ile Phe Thr Phe Leu Tyr Val Gln Met Arg Lys Thr Leu Arg
 225 230 235 240

20 Phe Arg Glu Gln Arg Tyr Ser Leu Phe Lys Leu Val Phe Ala Val Met
 245 250 255

Val Val Phe Leu Leu Met Trp Ala Pro Tyr Asn Ile Ala Phe Phe Leu
 260 265 270

25 Ser Thr Phe Lys Glu His Phe Ser Leu Ser Asp Cys Lys Ser Ser Tyr
 275 280 285

Asn Leu Asp Lys Ser Val His Ile Thr Lys Leu Ile Ala Thr Thr His
 30 290 295 300

Cys Cys Ile Asn Pro Leu Leu Tyr Ala Phe Leu Asp Gly Thr Phe Ser
 305 310 315 320

35 Lys Tyr Leu Cys Arg Cys Phe His Leu Arg Ser Asn Thr Pro Leu Gln
 325 330 335

Pro Arg Gly Gln Ser Ala Gln Gly Thr Ser Arg Glu Glu Pro Asp His
 40 340 345 350

Ser Thr Glu Val
 355

45 (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 355 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 50 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

60 Met Glu Thr Pro Asn Thr Thr Glu Asp Tyr Asp Thr Thr Glu Phe
 1 5 10 15

Asp Tyr Gly Asp Ala Thr Pro Cys Gln Lys Val Asn Glu Arg Ala Phe
 20 25 30

	Gly Ala Gln Leu Leu Pro Pro Leu Tyr Ser Leu Val Phe Val Ile Gly			
	35	40	45	
5	Leu Val Gly Asn Ile Leu Val Val Leu Val Leu Val Gln Tyr Lys Arg			
	50	55	60	
	Leu Lys Asn Met Thr Ser Ile Tyr Leu Leu Asn Leu Ala Ile Ser Asp			
	65	70	75	80
10	Leu Leu Phe Leu Phe Thr Leu Pro Phe Trp Ile Asp Tyr Lys Leu Lys			
	85	90	95	
	Asp Asp Trp Val Phe Gly Asp Ala Met Cys Lys Ile Leu Ser Gly Phe			
	100	105	110	
15	Tyr Tyr Thr Gly Leu Tyr Ser Glu Ile Phe Phe Ile Ile Leu Leu Thr			
	115	120	125	
20	Ile Asp Arg Tyr Leu Ala Ile Val His Ala Val Phe Ala Leu Arg Ala			
	130	135	140	
	Arg Thr Val Thr Phe Gly Val Ile Thr Ser Ile Ile Ile Trp Ala Leu			
	145	150	155	160
25	Ala Ile Leu Ala Ser Met Pro Gly Leu Tyr Phe Ser Lys Thr Gln Trp			
	165	170	175	
	Glu Phe Thr His His Thr Cys Ser Leu His Phe Pro His Glu Ser Leu			
	180	185	190	
30	Arg Glu Trp Lys Leu Phe Gln Ala Leu Lys Leu Asn Leu Phe Gly Leu			
	195	200	205	
	Val Leu Pro Leu Leu Val Met Ile Ile Cys Tyr Thr Gly Ile Ile Lys			
35	210	215	220	
	Ile Leu Leu Arg Arg Pro Asn Glu Lys Lys Ser Lys Ala Val Arg Leu			
	225	230	235	240
40	Ile Phe Val Ile Met Ile Ile Phe Phe Leu Phe Trp Thr Pro Tyr Asn			
	245	250	255	
	Leu Thr Ile Leu Ile Ser Val Phe Gln Asp Phe Leu Phe Thr His Glu			
	260	265	270	
45	Cys Glu Gln Ser Arg His Leu Asp Leu Ala Val Gln Val Thr Glu Val			
	275	280	285	
50	Ile Ala Tyr Thr His Cys Cys Val Asn Pro Val Ile Tyr Ala Phe Val			
	290	295	300	
	Gly Glu Arg Phe Arg Lys Tyr Leu Arg Gln Leu Phe His Arg Arg Val			
	305	310	315	320
55	Ala Val His Leu Val Lys Trp Leu Pro Phe Leu Ser Val Asp Arg Leu			
	325	330	335	
	Glu Arg Val Ser Ser Thr Ser Pro Ser Thr Gly Glu His Glu Leu Ser			
	340	345	350	
60	Ala Gly Phe			
	355			

(2) INFORMATION FOR SEQ ID NO:14:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 374 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: protein

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Leu Ser Thr Ser Arg Ser Arg Phe Ile Arg Asn Thr Asn Glu Ser
1 5 10 15

Gly Glu Glu Val Thr Thr Phe Phe Asp Tyr Asp Tyr Gly Ala Pro Cys
20 25 30

20 His Lys Phe Asp Val Lys Gln Ile Gly Ala Gln Leu Leu Pro Pro Leu
35 40 45

25 Tyr Ser Leu Val Phe Ile Phe Gly Phe Val Gly Asn Met Leu Val Val
50 55 60

Leu Ile Leu Ile Asn Cys Lys Lys Leu Lys Cys Leu Thr Asp Ile Tyr
65 70 75 80

30 Leu Leu Asn Leu Ala Ile Ser Asp Leu Leu Phe Leu Ile Thr Leu Pro
85 90 95

Leu Trp Ala His Ser Ala Ala Asn Glu Trp Val Phe Gly Asn Ala Met
35 100 105 110

Cys Lys Leu Phe Thr Gly Leu Tyr His Ile Gly Tyr Phe Gly Gly Ile
115 120 125

40 Phe Phe Ile Ile Leu Leu Thr Ile Asp Arg Tyr Leu Ala Ile Val His
130 135 140

Ala Val Phe Ala Leu Lys Ala Arg Thr Val Thr Phe Gly Val Val Thr
45 145 150 155 160

145 Ser Val Ile Thr Trp Leu Val Ala Val Phe Ala Ser Val Pro Gly Ile
165 170 175

Ile Phe Thr Lys Cys Gln Lys Glu Asp Ser Val Tyr Val Cys Gly Pro
50 180 185 190

55 Tyr Phe Pro Arg Gly Trp Asn Asn Phe His Thr Ile Met Arg Asn Ile
195 200 205

Leu Gly Leu Val Leu Pro Leu Leu Ile Met Val Ile Cys Tyr Ser Gly
55 210 215 220

Ile Leu Lys Thr Leu Leu Arg Cys Arg Asn Glu Lys Lys Arg His Arg
225 230 235 240

60 Ala Val Arg Val Ile Phe Thr Ile Met Ile Val Tyr Phe Leu Phe Trp
245 250 255

Thr Pro Tyr Asn Ile Val Ile Leu Leu Asn Thr Phe Gln Glu Phe Phe
260 265 270

Gly Leu Ser Asn Cys Glu Ser Thr Ser Gln Leu Asp Gln Ala Thr Gln
 275 280 285
 5 Val Thr Glu Thr Leu Gly Met Thr His Cys Cys Ile Asn Pro Ile Ile
 290 295 300
 Tyr Ala Phe Val Gly Glu Lys Phe Arg Ser Leu Phe His Ile Ala Leu
 305 310 315 320
 10 Gly Cys Arg Ile Ala Pro Leu Gln Lys Pro Val Cys Gly Gly Pro Gly
 325 330 335
 Val Arg Pro Gly Lys Asn Val Lys Val Thr Thr Gln Gly Leu Leu Asp
 15 340 345 350
 Gly Arg Gly Lys Gly Lys Ser Ile Gly Arg Ala Pro Glu Ala Ser Leu
 355 360 365
 20 Gln Asp Lys Glu Gly Ala
 370

(2) INFORMATION FOR SEQ ID NO:15:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 355 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 30 (ii) MOLECULE TYPE: protein

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
 Met Thr Thr Ser Leu Asp Thr Val Glu Thr Phe Gly Thr Thr Ser Tyr
 1 5 10 15
 40 Tyr Asp Asp Val Gly Leu Leu Cys Glu Lys Ala Asp Thr Arg Ala Leu
 20 25 30
 Met Ala Gln Phe Val Pro Pro Leu Tyr Ser Leu Val Phe Thr Val Gly
 45 35 40 45
 Leu Leu Gly Asn Val Val Val Met Ile Leu Ile Lys Tyr Arg Arg
 50 55 60
 50 Leu Arg Ile Met Thr Asn Ile Tyr Leu Leu Asn Leu Ala Ile Ser Asp
 65 70 75 80
 Leu Leu Phe Leu Val Thr Leu Pro Phe Trp Ile His Tyr Val Arg Gly
 85 90 95
 55 His Asn Trp Val Phe Gly His Gly Met Cys Lys Leu Leu Ser Gly Phe
 100 105 110
 60 Tyr His Thr Gly Leu Tyr Ser Glu Ile Phe Phe Ile Ile Leu Leu Thr
 115 120 125
 Ile Asp Arg Tyr Leu Ala Ile Val His Ala Val Phe Ala Leu Arg Ala
 130 135 140

Arg Thr Val Thr Phe Gly Val Ile Thr Ser Ile Val Thr Trp Gly Leu
 145 150 155 160

5 Ala Val Leu Ala Ala Leu Pro Glu Phe Ile Phe Tyr Glu Thr Glu Glu
 165 170 175

Leu Phe Glu Glu Thr Leu Cys Ser Ala Leu Tyr Pro Glu Asp Thr Val
 180 185 190

10 Tyr Ser Trp Arg His Phe His Thr Leu Arg Met Thr Ile Phe Cys Leu
 195 200 205

15 Val Leu Pro Leu Leu Val Met Ala Ile Cys Tyr Thr Gly Ile Ile Lys
 210 215 220

Thr Leu Leu Arg Cys Pro Ser Lys Lys Lys Tyr Lys Ala Ile Arg Leu
 225 230 235 240

20 Ile Phe Val Ile Met Ala Val Phe Phe Ile Phe Trp Thr Pro Tyr Asn
 245 250 255

Val Ala Ile Leu Leu Ser Ser Tyr Gln Ser Ile Leu Phe Gly Asn Asp
 260 265 270

25 Cys Glu Arg Ser Lys His Leu Asp Leu Val Met Leu Val Thr Glu Val
 275 280 285

Ile Ala Tyr Ser His Cys Cys Met Asn Pro Val Ile Tyr Ala Phe Val
 290 295 300

30 Gly Glu Arg Phe Arg Lys Tyr Leu Arg His Phe Phe His Arg His Leu
 305 310 315 320

Leu Met His Leu Gly Arg Tyr Ile Pro Phe Leu Pro Ser Glu Lys Leu
 325 330 335

Glu Arg Thr Ser Ser Val Ser Pro Ser Thr Ala Glu Pro Glu Leu Ser
 340 345 350

40 Ile Val Phe
 355

(2) INFORMATION FOR SEQ ID NO:16:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 360 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: protein

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Asn Pro Thr Asp Ile Ala Asp Thr Thr Leu Asp Glu Ser Ile Tyr
 1 5 10 15

60 Ser Asn Tyr Tyr Leu Tyr Glu Ser Ile Pro Lys Pro Cys Thr Lys Glu
 20 25 30

	Gly	Ile	Lys	Ala	Phe	Gly	Glu	Leu	Phe	Leu	Pro	Pro	Leu	Tyr	Ser	Leu
	35						40						45			
5	Val	Phe	Val	Phe	Gly	Leu	Leu	Gly	Asn	Ser	Val	Val	Val	Leu	Val	Leu
	50						55						60			
	Phe	Lys	Tyr	Lys	Arg	Leu	Arg	Ser	Met	Thr	Asp	Val	Tyr	Leu	Leu	Asn
	65						70					75				80
10	Leu	Ala	Ile	Ser	Asp	Leu	Leu	Phe	Val	Phe	Ser	Leu	Pro	Phe	Trp	Gly
								85			90			95		
	Tyr	Tyr	Ala	Ala	Asp	Gln	Trp	Val	Phe	Gly	Leu	Gly	Leu	Cys	Lys	Met
							100			105			110			
15	Ile	Ser	Trp	Met	Tyr	Leu	Val	Gly	Phe	Tyr	Ser	Gly	Ile	Phe	Phe	Val
							115			120			125			
20	Met	Leu	Met	Ser	Ile	Asp	Arg	Tyr	Leu	Ala	Ile	Val	His	Ala	Val	Phe
							130			135			140			
	Ser	Leu	Arg	Ala	Arg	Thr	Leu	Thr	Tyr	Gly	Val	Ile	Thr	Ser	Leu	Ala
							145			150			155			160
25	Thr	Trp	Ser	Val	Ala	Val	Phe	Ala	Ser	Leu	Pro	Gly	Phe	Leu	Phe	Ser
							165			170			175			
	Thr	Cys	Tyr	Thr	Glu	Arg	Asn	His	Thr	Tyr	Cys	Lys	Thr	Lys	Tyr	Ser
							180			185			190			
30	Leu	Asn	Ser	Thr	Thr	Trp	Lys	Val	Leu	Ser	Ser	Leu	Glu	Ile	Asn	Ile
							195			200			205			
35	Leu	Gly	Leu	Val	Ile	Pro	Leu	Gly	Ile	Met	Leu	Phe	Cys	Tyr	Ser	Met
							210			215			220			
	Ile	Ile	Arg	Thr	Leu	Gln	His	Cys	Lys	Asn	Glu	Lys	Lys	Asn	Lys	Ala
							225			230			235			240
40	Val	Lys	Met	Ile	Phe	Ala	Val	Val	Val	Leu	Phe	Leu	Gly	Phe	Trp	Thr
							245			250			255			
	Pro	Tyr	Asn	Ile	Val	Phe	Leu	Glu	Thr	Leu	Val	Glu	Leu	Glu	Val	
							260			265			270			
45	Leu	Gln	Asp	Cys	Thr	Phe	Glu	Arg	Tyr	Leu	Asp	Tyr	Ala	Ile	Gln	Ala
							275			280			285			
50	Thr	Glu	Thr	Leu	Ala	Phe	Val	His	Cys	Cys	Leu	Asn	Pro	Ile	Ile	Tyr
							290			295			300			
	Phe	Phe	Leu	Gly	Glu	Lys	Phe	Arg	Lys	Tyr	Ile	Leu	Gln	Leu	Phe	Lys
							305			310			315			320
55	Thr	Cys	Arg	Gly	Leu	Phe	Val	Leu	Cys	Gln	Tyr	Cys	Gly	Leu	Leu	Gln
							325			330			335			
	Ile	Tyr	Ser	Ala	Asp	Thr	Pro	Ser	Ser	Ser	Tyr	Thr	Gln	Ser	Thr	Met
							340			345			350			
60	Asp	His	Asp	Leu	His	Asp	Ala	Leu								
							355			360						

(2) INFORMATION FOR SEQ ID NO:17:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTAATGATCA GTCAACGGGG GAC

23

15 (2) INFORMATION FOR SEQ ID NO:18:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CCAGCAAGCT TGCAACCTTA ACCA

24

(2) INFORMATION FOR SEQ ID NO:19:

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: peptide

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Asp Tyr Lys Asp Asp Asp Asp Lys Leu
1 5

45

WHAT IS CLAIMED IS:

1. A substantially pure or isolated polypeptide comprising a segment exhibiting sequence homology to a 5 corresponding portion of a mature protein selected from the group consisting of:

- i) TECK;
- ii) MIP-3 α ;
- iii) MIP-3 β ;
- 10 iv) DC CR; and
- v) M/DC CR;

wherein said homology is at least about 70% identity and said portion is at least about 25 amino acid residues.

15 2. The polypeptide of claim 1, further comprising a second segment exhibiting:

- a) at least about 90% identity over at least 9 amino acid residues; or
- b) at least about 80% identity over at least 17 20 amino acid residues.

3. The polypeptide of either claim 1 or 2, selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10 and 25 SEQ ID NO: 12.

4. An isolated or recombinant nucleic acid which encodes the polypeptides of any of claims 1-3.

30 5. The nucleic acid of claim 4, selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11.

35 6. A nucleic acid which hybridizes under stringent wash conditions of 55° C and less than 150 mM salt to a nucleic acid of claim 4 or 5.

7. The nucleic acid of claim 6, which further exhibits at least about 85% identity over a stretch of at

least about 30 nucleotides to a nucleic acid of claim 4 or 5.

8. A vector comprising a nucleic acid of any of
5 claims 4-7.

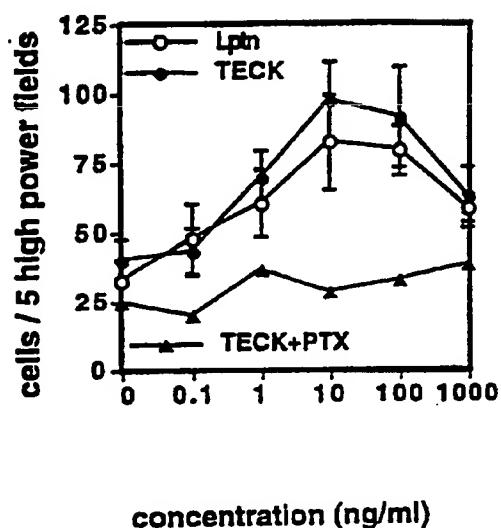
9. A host cell comprising a nucleic acid or vector
of any of claims 4-8.

10 10. A method for making a polypeptide comprising
culturing a host cell of claim 9 under conditions in
which the nucleic acid or vector is expressed.

11. 11. A binding compound comprising an antibody or
15 antigen binding fragment therefrom which binds to a
polypeptide of any of Claims 1-3.

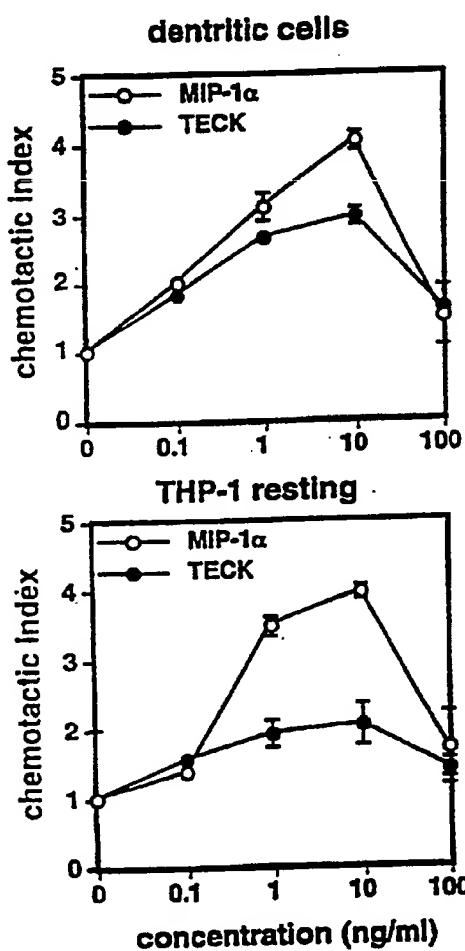
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1A

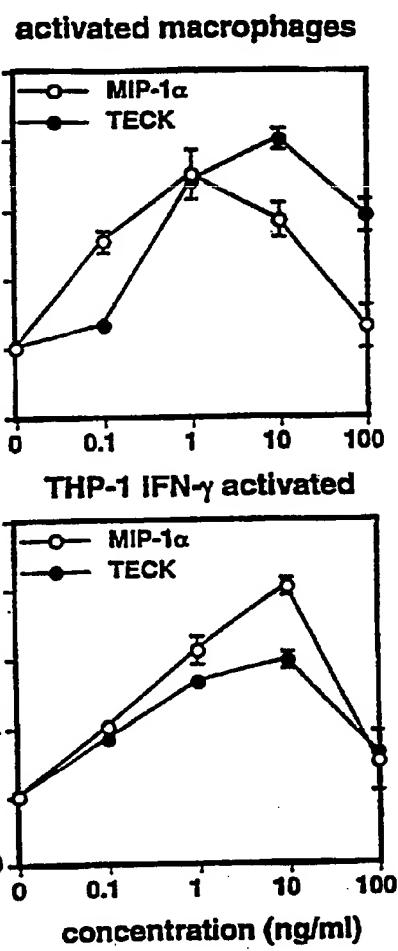


SUBSTITUTE SHEET (RULE 26)

2A



2B



2C

2D